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First isolation of *Toxoplasma gondii* from rodents and stray cats in northeast of Iran

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ABSTRACT

Toxoplasma gondii is a common coccidian parasite with stray cat family as definitive host, and a wide range of warm blooded animals and human as intermediate hosts. The objective of this study was to isolate *T. gondii* from rodents and stray cats in northeast of Iran. Genomic DNA was extracted from 30–50 mg of the rodents and stray cats' tissue using phenol-chloroform extraction method. Using PCR-RFLP, the genes (SAG1, SAG3, GRA6, 18SrRNA) and sequencing method, the isolation of *T. gondii* from these hosts was determined. ELISA method was also used on serum samples to evaluate the IgG antibodies against *T. gondii* among the hosts. Out of 286 rodents and 210 stray cats tested, the DNA of *T. gondii* was detected in 68 and 38 samples, respectively. Nucleotide sequencing and phylogenetic analysis confirmed the RFLP results. It was also observed that dominant genotype of T. *gondii* in infected rodents and stray cats belonged to genotype 3. The results of this study indicated that rodent and stray cat hosts can play an important role in maintaining the *Toxoplasma* transmission cycle in the Golestan province.

Key words: Iran, MLST, Rodents, Stray cats, *Toxoplasma gondii*

Toxoplasma gondii is a parasite that has been studied extensively due to its importance in medicine, veterinary medicine as well as its availability. Various strains of *T. gondii* are divided into low-intensity and high-intensity strains. Of course, most strains are separated from nature in a very low-density group. The strains RH, Cj, Ang, Toxo ENT, Toxo P are of high-end types and the strains ME49, Beverly, Tehran etc. are of low-frequency species (Anvari *et al.* 2018, Zhou *et al.* 2018a, Zhou *et al.* 2018b). Genetic diversity and population structure are strongly influenced by proliferation of the sexual recombination and the evolutionary selection of the recombinant species. Proliferation without sexual recombination leads to clonal populations. Despite of such a structure, it is expected that the same genotypes to be spread in large geographic areas at several-year intervals (Biradar *et al.* 2014, Silva *et al.* 2018, Skorpikova *et al.* 2018). The epidemiological structure of the infectious diseases such as toxoplasmosis is determined by the genotypic studies. In this regard, a number of unpredictable multicellular genotypes will appear along with a number of dominant genotypes (Cai *et al.* 2015, Salman *et al.* 2018, Shaddel *et al.* 2018). The aim of this study was to carry out a multi locus sequence typing (MLST) of *T. gondii* in the presence of four genes to detect this protozoa in rodents and stray cats, in northeast of Iran. The necessity of this study was to investigate the genotypes of *T. gondii,* based on the serological and molecular methods.

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MATERIALS AND METHODS

Study area: Golestan province is divided into five geographic regions, viz. North, South, East, West and Center. The area has sub-tropical and semi-arid condition. The collection of the rodent specimens was carried out during night hunting and clearing of rodents in different areas of Golestan province, with coordination of municipality and Environment Protection Organization.

Ethics and participation consent: This study was approved by Tarbiat Modares University Ethics Committee.

Sampling: Wild rodents (286) and stray cats' (210) tissue (brain and heart) were collected from Golestan province in 2017. The samples were obtained using intravenous blood sampling method with syringe followed by killing the animal in order to isolate their tissues. After transferring the rodents to the laboratory, they were sanitized and sterilized as much as possible, and separated immediately. The specimens were kept at –20°C for freezing until their brain was isolated. The hosts' hearts were also separated, after being numbered according to their heads and placed in a freezer at –20°C.

Hosts typing: Rattus rattus also known as black mouse, rodent mouse, has tail longer than the head and body. They

are seen in black or dark gray colours. Their weight is about 30–70 g with the body length of 14 to 17 cm. The animal lives throughout the coast of the Caspian Sea, Persian Gulf, Oman Sea, Tehran and East Azerbaijan. *Rattus norvegicus* (Portrait rat, brown rat, rat and storage mouse) has body weight of 200 to 400 g and body length of 22 to 26 cm. This is not a native rat inIran and probably has arrived in Iran by ships. *Mus musculus* is a narrow and small mouse with large eyes and fairly large oyster loin, weighing 10 to 30 g. Its body length is about 6 to 12 cm. The length of the tail equals to the length of the head and body, covered with fine hairs. It is grey with brownish to brown back. *Rombo mysopimus* is a small and fairly brown rod, which lives in a rural leishmaniosis reservoir. *Stray cats*, were very clear in terms of appearance and require no description.

ELISA test: ELISA method was used on serum samples to test the IgG antibodies against *T. gondii* in these hosts. For this experiment, a Euro-Immunekit was used. The procedure was carried out in accordance with the manufacturer's instructions. Positive control in the ELISA test was embedded in the commercial kit.

DNA extraction: DNA extraction was conducted using phenol-chloroform method on the tissue samples of the hosts within 2 h. The samples were kept at –20°C in freezer until the DNA extraction. Tehran strain of *T. gondii* served as positive control .

Evaluation of DNA quality: To evaluate the quality of DNA, the ratio of the absorption of the pure DNA sample at wavelengths 260 and 280 nanometers was estimated (be equal to or greater than 1.8; and the ratio of less than 1.8 means presence of protein impurities in the sample).

Amplification of the four genes of T. gondii (*Polymerase chain reaction*): Four gene primers were purchased from Pishgam Company-Iran to detect DNA of *T. gondii* (Table1). The reason for using these genes was generalization of the molecular markers in various strains of *T. gondii*. The PCR kit was also purchased from the Pishgam Company.

Restriction fragment length polymorphism (RFLP): First, mix 10 µl of the second PCR product with 1 µl of RFLP enzyme and 2 µl of enzyme buffer; then, the sterile distilled water volume was reached to 30 µl and incubated for 5 min at 65°C. The whole production was loaded into a 3% gel hole, in order to observe the enzymatic cutting process. The statistical analysis was conducted using SPSS18 software.

Sequencing (*MLST*): For this purpose, 25 µl of the PCR product was electrophoresed on agarose gel for 45 min, until the PCR product was gently opened. The bands were cut from the gel using the transluminator and surgical blade. Glued bands were transferred to 1.5 microns. After measuring the gel weight, the remaining steps were performed according to the instructions of the Prime Prep ™ Gel Purification kit.

Statistical analysis: SPSS software was used for data analysis.

Table 1. Primer sequences

Gene	Primer sequence (5'–3')	Primer
SAG1	GCTGTAACATTGAGC TCCTTGASTTCCTG	Forward
SAG1	CCGGAACAGTACT GATTGTTGTCTTCTG	Reverse
SAG3	ATGCAGCTGTGGCGGCGCAG	Forward
SAG3	TTAGGCAGCCACATGCACAAG	Reverse
GRA6	GTAGCGTGCTTGTTGGCGAC	Forward
GRA6	TACAAGACATAGAGTGCCCC	Reverse
18s rRNA	CCATGCATGTCTAAGTATAAGC	Forward
18s rRNA	GGCAAATGCTTTCGCAGTAG	Reverse

RESULTS AND DISCUSSION

Out of 130 *R. rattu* samples, 34 were positive for all four genes of *T. gondii*. Similarly, out of 46 *R. norvegicus* samples, 11; of the 60 *M. musculus* samples, 9 samples and of the 50 *Rombo mysopimus* samples, 5 were positive for all four genes *T. gondii* respectively. Positive samples showed 1180 bp band for SAG1 gene, 311 bp for SAG3 gene, 351 bp for GRA6 gene and 700 bp for 18s rRNA.

The dominant genotype 3 of *T. gondii* was observed in the simultaneous sampling of rodents and stray cats of Golestan province (Table 2).

Also, 180 stray cats were positive according to the ELISA IgG antibody test (epidemiological importance) while 38 stray cats were positive according to the PCR test (Genotyping importance). The highest prevalence of *Toxoplasma gondii* among rodents was observed in *R. rattus* samples (29%) followed by *R. novegicus* (26.6%), *M. musculus* (16.6%) and *R. mysopimus* (16%). About 18.1% stray cat samples were positive for *T. gondii*. All the above results were obtained using SAG1, SAG3, GRA6 and 18S rRNA genes.

The prevalence of toxoplasmosis was higher by serological method than by molecular method. In addition, the dominant genotype of *T. gondii* in both hosts was type-3 in northeast of Iran.

Based on the sequencing results of brain and heart tissue samples of both host species using four genes (Table 3), it can be concluded that using the PCR-RFLP method as well

Table 2. Frequency distribution of *Toxoplasma* genotypes among the wild rodents and stray cats in northeast of Iran

Host	Numbers	Positive rate for PCR	Positive rate for ELISA	Genotype 3	Genotype 2
Rattus rattus	131	38/131 (29%)	120/131 (91.6%)	29/38 (76.3%)	9/38 (23.4%)
Rattus novegicus	45	12/45 (26.6%)	39/45 (86.6%)	8/12 (66.6%)	4/12 (33.4%)
Mus musculus	60	10/60 (16.66%)	49/60 (81.6%)	7/10 (70%)	3/10 (30%)
Rombo mysopimus	50	8/50 (16%)	38/50 (76%)	6/8 (75%)	2/8 (25%)
Stray cat	210	38/210 (18%)	180/210 (85.7%)	30/38 (78.9%)	8/38 (21.1%)

as the above genes, complete overlapping for genotype 2 and 3, can be distinguished. An important point in this study was that none of the samples studied in this study were genotype 1 or RH strain. The positive cases observed with PCR and a number of specimens were sent to the Pishgam Company, using each of the four genes and considering the rodent and cat species, which covers almost all of our hosts, to determine the sequence. The results obtained from them at the NCBI base, PUBMED, BLAST and all confirmed the positive results of the molecular method in the laboratory. The sequencing results, alignment and phylogenetic tree mapping with Mega 7 software.

Table 3. Comparative Sanger sequencing results on wild rodents and stray cats

Tissue type	Host	GRA6 RFLP result	SAG3 RFLP result	SAG1 RFLP result	Accession number
Brain	*rodent*	2	2	2	LC412908
Brain	*rodent*	3	3	3	LC414528
Brain	*rodent*	3	3	3	LC414529
Brain	*rodent*	3	3	3	LC414530
Brain	*rodent*	3	3	3	LC412903
Brain	*rodent*	3	3	3	LC414531
Brain	*rodent*	2	2	2	LC414532
Brain	*rodent*	3	3	3	LC414533
Brain	*rodent*	3	3	3	LC414534
Brain	*rodent*	3	3	3	LC411944
Brain	*rodent*	3	3	3	LC414526
Brain	*rodent*	2	2	2	LC414527
Heart	*Stray cat*	3	3	3	LC416237
Heart	*Stray cat*	2	2	2	LC416238

These accession numbers, are the results of sequence determination, which approved using the Sangerin www.ddbj.com GENE BANK (Table 4).

Phylogenetic trees of the known genotypes of *T. gondii* with the comparison of *Toxoplasma* isolates among the rodents and stray cats are depicted in Figs 1–2. Identifications in this study were based on GRA6 and 18s rRNA genes, using the Neighbor-Joining algorithm and 1,000 repetitions. These phylogenetic trees showed that the circulating genotypes of *T. gondii* among the rodents and stray cats in Northeast of Iran were genotypes 3 and 2.

One of the main difficulties of this study was simultaneous sampling of the rodents and stray cats. Genotyping biomarkers have been developed to differentiate *Toxoplasma* isolates. They are suitable for studies of molecular epidemiology and genetic population structure of *T. gondii*. These markers include multistage multiplicity multiplication of polymerase chain reaction (RAPD-PCR), multicellular enzyme electrolysis (MLEE), microsatellite analysis and sequencing of DNA molecules and genotyping by sociological method (Yasodhara *et al.* 2001, Ahmadpour *et al.* 2017, Kalambhe *et al.* 2017, Ebrahimzadeh *et al.* 2018, Rocha *et al.* 2018, Roux *et al.* 2018). Multiplex PCR-RFLP is widely used in genetic studies of *T. gondii* due to its cost-effectiveness and ease of use. This method is based on the effect of endonuclease enzymes to detect SNPs among the digested DNA sequences of PCR product and to detect DNA distinct patterns by electrophoresis on agarose gel. In Iran, serological methods for detecting *T. gondii* antibodies are commonly used in cat. It is likely that the low level of evaluation of the *Toxoplasma* infection reported in the molecular method is related to the absence of tissue cysts in various tissue samples (Battisti *et al.* 2018, Bigal *et al.* 2018, Pipia *et al.* 2018). It should be noted that even in positive cases reported with the serology method do not reveal the presence of *T. Gondii* DNA, and that serology methods in this regard will not be reliable for estimating the infection rate (Borkakoty *et al.* 2016, Wang *et al.* 2018b, Zhang *et al.* 2018b). According to Sharif *et al.* (2009) in Sari, the rate of infection was 40% with the same method; and in all of these serological methods the host cat had been rejected. But *Toxoplasma* infection in stray cats of Sari is lower than that of Golestan province. Saki and his colleagues from Ahvaz reported 6% of the 100 rats using GRA6 gene. Also, environmental factors such as the climate are likely to be effective in protecting the contamination caused by the cysts in the environmental cycle (Saki and Khademvatan 2014). According to the reports, contamination of *T. gondii* in the temperate region (33–80%) is higher than the prevalence in hot and humid regions of southern Iran (20–35%), and it can be concluded that the higher contamination in the Golestan province area has led to more contamination of the rodents and stray cats. In this study, the positive results of Toxoplasmosis by ELISA method were much higher than those reported in other regions of Iran.

Yera and colleagues studied the new genotypes of *T. gondii* parasites in the Polynesia region, using PCR-RFLP method; and discovered some atypical genotypes (Yera *et al.* 2014). In 2014, Dodd *et al.* (2004) reviewed the genotype of *T. gondii* by PCR-RFLP and SAG genes on the *Pipistrellus pipistrellus* and *P. pygmaeus* specimens, which contained 83% of genotype 1. Higa *et al.* (2014) reviewed the genotype of *T. gondii* parasite, using PCR-RFLP and SAG, GRA6, and BTUB genes on pregnant mothers; two of them had TOXO DB166 genotype, and we also obtained a new genotype of this parasite in Northeast of Iran. Schwab *et al.* (2014) reviewed the genotype of *T. gondii* parasite using the PCR-RFLP method and concluded that genotypes 2 and 3 in Africa, genotypes 9 and 10 in Asia, and genotypes 1, 2, 3 were dominant in Europe. Gomez-Samblas *et al.* (2015) applied the qPCR to detect toxoplasmosis in pigs,

Table 4. Accession numbers of the first isolations of *T. gondii* from Northeast of Iran

LC412908	LC414528	LC414529	LC414530	LC412903	LC414531	LC414532
LC414533	LC414534	LC411944	LC414526	LC414527	LC416237	LC416238

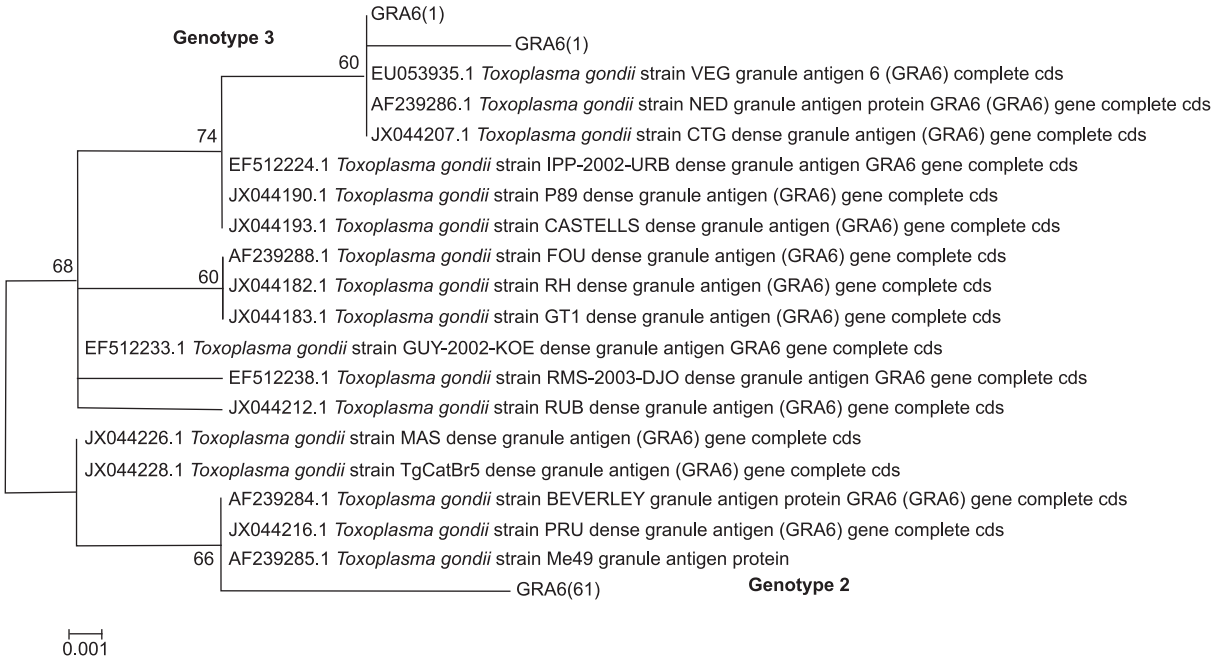

Fig. 1. Phylogenetic tree of *T. gondii* in the presence of GRA6 gene.

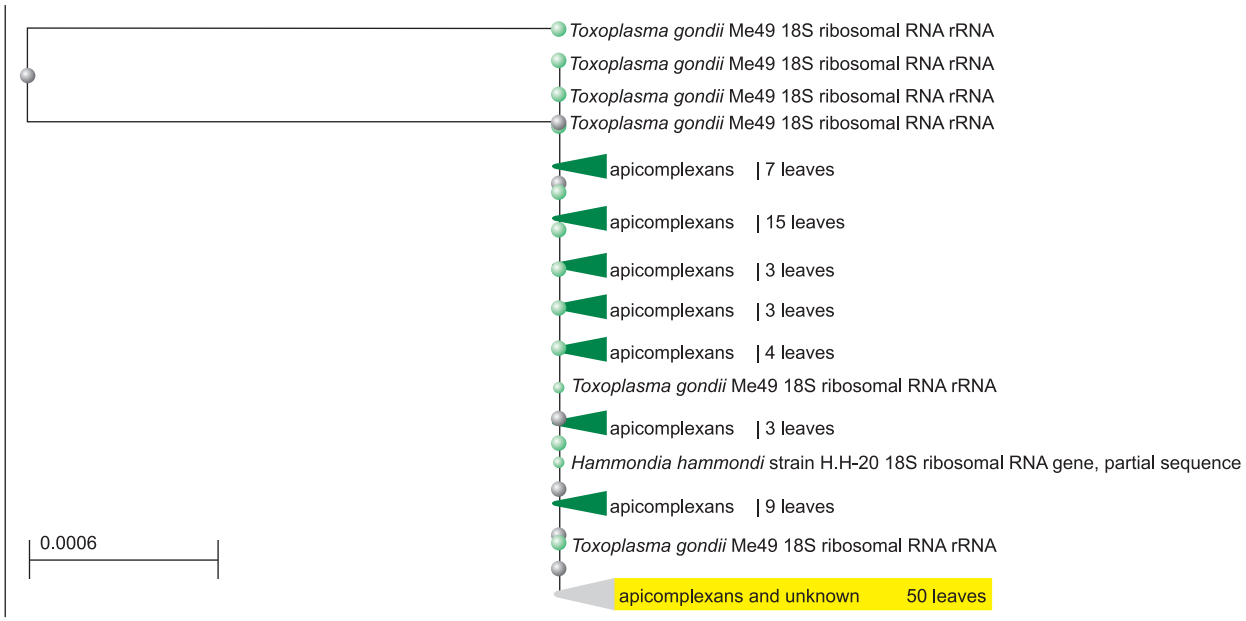

Fig. 2. Phylogenetic tree of *T. gondii* in the presence of 18s rRNA gene.

and reported about 35% of cases; however, in this study, the prevalent genotype of *Toxoplasma* in northeast of Iran was a subset of type 2 and 3.

In recent years, many genotyping studies of toxoplasmosis have been conducted and various genotypes had been reported in different geographic regions (Wang *et al.* 2018a, Zhang *et al.* 2018a). Moreover, many serological and genotyping studies had been conducted on *T. gondii* in India, as well as prokaryotic expression and molecular characterization of surface antigen 3 (SAG 3) protein and GRA5 of *T. gondii*. However, these results differed from those reported in ours, due to the genotype distribution of *Toxoplasma* in different parts of the world (Singh *et al.* 2011, Sudan *et al.* 2014).

As shown earlier, clonal genotypes are dominant in the Middle East, and the results of our study also confirm this. The genotypic similarity between the hosts studied in Golestan province indicates the maintenance of *Toxoplasma* cycles among these hosts. As a result, the presence of these hosts in the human environment can be a risk factor for the

transmission of infection to humans. Most importantly, the role of soil contaminated with cysts can be pointed out, because according to the dominant genotype in circulation (genotype 3) among the hosts of the Golestan province rust, it can be argued that the source of contamination is the same. In this study, the most genotypes derived from the four species of rodents and stray cats were type 3 and in some samples, genotype 2 was observed with a lower percentage, indicating that these two genotypes were dominant in the wildlife of the Golestan province area. Importantly, in the samples studied in this study, none of the rodents and stray cats harboured genotype 1 or RH strain.

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REFERENCES

Ahmadpour G R, Ezatpour B, Hadighi R, Oormazdi H, Akhlaghi L, Tabatabaei F, Azami M, Nejad M M and Mahmoudvand H. 2017. Seroepidemiology of *Toxoplasma gondii* infection in pregnant women in west Iran: determined by ELISA and PCR analysis. *Journal of Parasitic Diseases* **41**(1): 237–42.

Anvari D, Saadati D, Nabavi R and Alipour M E. 2018. Epidemiology and molecular prevalence of *Toxoplasma gondii* in cattle slaughtered in Zahedan and Zabol districts, South East of Iran. *Iran Journal of Parasitology* **13**(1): 114–19.

Battisti E, Zanet S, Trisciuoglio A, Bruno S and Ferroglio E. 2018. Circulating genotypes of *Toxoplasma gondii* in North-western Italy. *Veterinary Parasitology* **253**: 43–47.

Bigal E, Morick D, Scheinin A P, Salant H, Berkowitz A, King R, Levy Y, Melero M, Sanchez-Vizcaino J M, Goffman O, Hadar N, Roditi-Elasar M and Tchernov D. 2018. Detection of *Toxoplasma gondii* in three common bottlenose dolphins (*Tursiops truncatus*). A first description from the Eastern Mediterranean Sea. *Veterinary Parasitology* **258**: 74–78.

Biradar S S, Saravanan B C, Tewari A K, Sreekumar C, Sankar M and Sudhakar N R. 2014. Genetic characterization of *Toxoplasma gondii* isolates from chickens in India by GRA6 gene sequence analysis. *Acta Parasitol* **59**(4): 666–74.

Borkakoty B, Biswas D, Jakharia A and Mahanta J. 2016. Seroprevalence of *Toxoplasma gondii* among pregnant women in North-east India. *Journal of the Association of Physicians of India* **64**(10): 24–28.

Cai Y, Wang Z, Li J, Li N, Wei F and Liu Q. 2015. Evaluation of an indirect ELISA using recombinant granule antigen GRA7 for serodiagnosis of *Toxoplasma gondii* infection in cats. *Journal of Parasitology* **101**(1): 37–40.

Dodd N S, Lord J S, Jehle R, Parker S, Parker F, Brooks D R and Hide G. 2014. *Toxoplasma gondii:* Prevalence in species and genotypes of British bats (*Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*). *Experimental Parasitology* **139**: 6–11.

Ebrahimzadeh A, Shahraki M K and Mohammadi A. 2018. Serological and molecular diagnosis of *Toxoplasma gondii* in patients with Schizophrenia. *Journal of Parasitic Diseases* **42**(2): 177–81.

Gomez-Samblas M, Vílchez S, Racero J, Fuentes M and Osuna A. 2015. Quantification and viability assays of *Toxoplasma gondii* in commercial "Serrano" ham samples using magnetic capture real-time qPCR and bioassay techniques. *Food Microbiology* **46**: 107–13.

Higa L T, Garcia J L, Su C, Rossini R C and Falavigna-Guilherme A L. 2014. *Toxoplasma gondii* genotypes isolated from pregnant women with follow-up of infected children in southern Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **108**(4): 244–46.

Kalambhe D, Gill J P S and Singh B B. 2017. Molecular detection of *Toxoplasma gondii* in the slaughter sheep and goats from North India. *Veterinary Parasitology* **241**: 35–38.

Pipia A P, Varcasia A, Dessi G, Panzalis R, Gai C, Nonnis F, Veronesi F, Tamponi C and Scala A. 2018. Seroepidemiological and biomolecular survey on *Toxoplasma gondii* infection on organic pig farms. *Parasitology Research* **117**(5): 1637–41.

Rocha D S, Nilsson M G, Maciel B M, Pena H F J, Alves B F, Silva A V, Gondim L F P and Albuquerque G R. 2018. Genetic diversity of *Toxoplasma gondii* isolates from free-range chickens in Bahia, Brazil. *Journal of Parasitology* **104**(4): 377–82.

Roux G, Varlet-Marie E, Bastien P, Sterkers Y and French N. 2018. National Reference Center for Toxoplasmosis. Evolution of Toxoplasma-PCR methods and practices: a French national survey and proposal for technical guidelines. *International Journal of Parasitology* **48**(9–10): 701–07.

Saki J and Khademvatan S. 2014. Detection of *Toxoplasma gondii* by PCR and mouse bioassay in rodents of Ahvaz District, Southwestern Iran. *BioMed Research International* Article ID # 383859.

Salman D, Pumidonming W, Oohashi E and Igarashi M. 2018. Prevalence of *Toxoplasma gondii* and other intestinal parasites in cats in Tokachi sub-prefecture, Japan. *Journal of Veterinary Medical Science* **80**(6): 960–67.

Shaddel M, Ebrahimi M and Tabandeh M R. 2018. Bioinformatics analysis of single and multi-hybrid epitopes of GRA-1, GRA-4, GRA-6 and GRA-7 proteins to improve DNA vaccine design against *Toxoplasma gondii*. *Journal of Parasitic Diseases* **42**(2): 269–76.

Sharif M, Daryani A, Nasrolahei M and Ziapour S P. 2009. Prevalence of *Toxoplasma gondii* antibodies in stray cats in Sari, northern Iran. *Tropical Animal Health and Production* **41**(2): 183–87.

Shwab E K, Zhu X Q, Majumdar D, Pena H F, Gennari S M, Dubey J P and Su C. 2014. Geographical patterns of *Toxoplasma gondii* genetic diversity revealed by multilocus PCR-RFLP genotyping. *Parasitology* **141**(4): 453–61.

Silva M A, Pena H F J, Soares H S, Aizawa J, Oliveira S, Alves B F, Souza D S, Melo R P B, Gennari S M, Mota R A and Silva J C R. 2018. Isolation and genetic characterization of *Toxoplasma gondii* from free-ranging and captive birds and mammals in Pernambuco state, Brazil. *Revista Brasileira Parasitologia Veterinaria* DoI: 10.1590/s1984-296120180059.

Singh H, Tewari A, Mishra A, Maharana B, Rao J and Raina O. 2011. Molecular cloning, comparative sequence analysis and prokaryotic expression of GRA5 protein of *Toxoplasma gondii*. *Indian Journal of Animal Sciences* **81**(3): 209–15.

Skorpikova L, Reslova N, Lorencova A, Plhal R, Drimaj J, Kamler J and Slany M. 2018. Molecular detection of *Toxoplasma gondii* in feathered game intended for human consumption in the Czech Republic. *International Journal of Food Microbiology* **286**: 75–79.

Sudan V, Tewari A K, Singh H and Sarvanan B. 2014. Prokaryotic expression and molecular characterization of surface antigen 3 (SAG 3) protein of *Toxoplasma gondii*. *Indian Journal of*

Animal Sciences **84**(8): 824–28.

Wang X L, Dong L, Zhang L, Lv Y, Li Q and Li H. 2018. Seroprevalence and genetic characterization of *Toxoplasma gondii* in naturally-infected synanthropic rodents in Yunnan Province, South-western China. *Journal of Parasitology* **104**(4): 383–87.

Wang X L, Dong L, Zhang L, Lv Y, Li Q and Li H L. 2018. Seroprevalence and genetic characterization of *Toxoplasma gondii* in naturally infected synanthropic rodents in Yunnan Province, South-western China. *Journal of Parasitology* **104**(4): 383–87.

Yasodhara P, Ramalakshmi B A and Sarma M K. 2001. A new approach to differentiate recent vs chronic *Toxoplasma* infection: avidity ELISA in *Toxoplasma* serology. *Indian Journal of Medical Microbiology* **19**(3): 145–48.

Yera H, Ajzenberg D, Lesle F, Eyrolle-Guignot D, Besnard M, Baud A, Georges A, Dupouy-Camet J and Dardé M L. 2014. New description of *Toxoplasma gondii* genotypes from French Polynesia. *Acta Tropica* **134**: 10–12.

Zhang X X, Jiang R L, Zhang N C, Wang C R, Tao W F, Xu P, Ma C F, Hou G and Ni H B. 2018. Molecular detection and genotyping of *Toxoplasma gondii* in free-ranging pigs in Northeastern China. *Infection, Genetics and Evolution* **63**: 110–15.

Zhang X X, Ren W X, Hou G, Liu Q, Yu T Q, Zhao Q and Ni H B. 2018. Seroprevalence and risk factors of *Toxoplasma gondii* infection in horses in Jilin Province and Inner Mongolia Autonomous Region, northern China. *Acta Tropica* **187**: 119–23.

Zhou Q, Wang Q, Shen H, Zhang Y, Zhang S, Li X and Acharya G. 2018. Seroepidemiological map of *Toxoplasma gondii* infection and associated risk factors in preconception period in China: A nationwide cross-sectional study. *Journal of Obstetrics and Gynaecology Research* **44**(6): 1134–39.

Zhou Z, Wu Y, Chen Y, Wang Z, Hu S, Zhou R, Dong C, Lin H and Nie K. 2018. Molecular and serological prevalence of *Toxoplasma gondii* and *Anaplasma* spp. infection in goats from Chongqing Municipality, China. *Parasite* **25**: 20.