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Molecular Detection and Genetic Characterization of *Toxoplasma gondii* using SAG1 & 18SrRNA Genes in Rodents of Golestan Province, Northeast of IRAN

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Review Article

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Abstract

Background: Toxoplasma parasite is from Toxoplasmatidea family that initially was seen in *Ctinodactylus Gondii* rodent. Toxoplasma parasites that extracted from different rodents are same in immunologic and morphologic characteristics but have differences in pathogenicity and genotypes in mice. The rodents are most reservoir host in environment that by attention of human environment vicinity to rodent's environment causes Toxoplasma dispersion in that area. The aim of this study was abundance detection of toxoplasmosis in rodents of golestan province using SAG1 and 18SrRNA genes.

Materials and methods: In this study we collected 285 rodents from Golestan forest and extracted brain and heart tissues to obtain DNA of SAG1 and 18SrRNA genes from these tissues. We divided these rodents to 4 groups and then detected the positive samples by PCR method.

Results: In this study we found 68 samples of these rodents were positive for SAG1 and 18SrRNA genes. 38 samples were Ratus ratus, 10 samples were Ratus norvegicus, 10samples were Mus musculus and 10 samples were Rombumys opimus.

Conclusion and Discussion: in this study we found that the different types of rodents were responsible to spread of toxoplasmosis, also SAG1 and 18SrRNA genes were very useful markers to detect toxoplasmosis in rodents of northeast area of IRAN.

Keywords: Toxoplasmosis; Sag1 and 18srrna Genes; Golestan Forest; Rodents Brain and Heart

Introduction

Toxoplasma gondii is an intracellular parasite that infected many hosts in IRAN, including human. Rodents, cats and domestic animals. Because domestic and feral cats are the natural definitive host, they play an important role in the extending of toxoplasmosis [1-5]. Rodents are very important reservoir in dissemination of toxoplasmosis in IRAN. The main tissues of rodents that infected by this parasite are brain and heart. Toxoplasmosis infection causes tissue cysts in these organs of rodents. The important genes that extracted from these organs to detect toxoplasmosis were SAG1 and 18SrRNA genes [5-10].

Toxoplasmosis infection pathway is eating of infected tissues from rodents by cats, then laying Oocysts from cats in environments to dissemination of infection to human or domestic animals. The major disease of this parasite in human is encephalitis or brain disorders [11-13]. Primary routes of acute human *T. gondii* infection include ingestion of tissue cysts in undercooked, contaminated meat, congenital infection through the placenta, and ingestion of oocysts from soil, water, or cat litter. Oocysts are produced by *T. gondii* only through sexual reproduction in its definitive host, the cat. Oocysts are shed in cat feces and can remain viable in soil and water samples for months to years. The infected rodents are the main food for cats and this cycle is the important cause of spreading the toxoplasmosis infection. My purpose was the abundance detection of toxoplasmosis in rodents of golestan province using SAG1 and 18SrRNA genes in brain and heart tissues [14-18].

Materials and Methods

Different regions of Golestan province have different climate and are notably heterogeneous. Northern parts are located in the arid and semi-arid climate, southern parts show a mountainous climate, and central and southern west parts have a moderate Mediterranean climate [Weather Centre Hashem] (Figure 1).

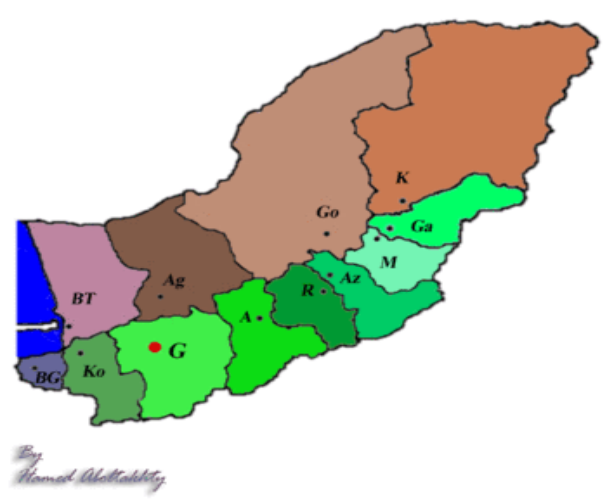

Figure1: AZ: Azadshahr, AG: Agh Ghala, G: Gorgan, BT: Bandar Torkaman

Histopathologic Examinations

We collected 285 mice from Golestan forest. To examine the infection status of visceral organ (the brain and heart) of mice were removed. This organ were fixed in 95% Ethanol and preserved in 4◦C until DNA extraction. These rodents divided in 4 groups: (*Rattus rattus, Rattus norvegicus, Mus musculus and Rombomys opimus*).

Rodents type	Numbers
Rattus rattus	130
Rattus norvegicus	45
Mus musculus	60
Rombomys opimus	50

Table1: The total samples of rodents divided in 4 groups.

DNA Extraction

Extraction of genomic DNA and genetic characterization Genomic DNA was extracted from approximately 3g of brain and heart tissues by sodium DNG/proteinase K method from Sinacolon company and eluted into 50 µl DDH2O according to the manufacturer's recommendations. A PCR targeting the *T. gondii* SAG1 and 18SrRNAgenes was performed to detect possible infection with *T. gondii*. DNA samples giving positive SAG1and 18SrRNA amplification were then used for genetic characterization *T. gondii.*

We cut 3g of tissue into small places and placed the samples into a 1.5ml sterile tube. Added 180 µl lyse buffer pepsin to homogenization and 400 ƛ (DNG/proteinase K) and homogenized the samples. If the sample size was larger than 3g we should increase the amount of lyse buffer proportionally.

Added 20µl proteinase K to the samples. Mixed immediately by mixing for 20 seconds. Incubated at 60◦c for 1hour to lyse the sample. If tissue was difficult to lyse, increased the incubation time to 2-3 hours. Mixed or inverted the samples every 10-15 minutes. Then we added 300ƛ isopropanol to DNA precipitation. After 5-10 minutes we washed the tubes by 70% ethanol and finally we eluted the DNA by DDH20. The purified DNA was evaluated in Nano drop system. The eluted DNA preserved in -20◦C freezer until using PCR method to detection of infection.

PCR analysis for T. gondii SAG1 and 18SrRNA genes

To genetically identify the presence of KI-1 Tachyzoites in visceral organs, PCR analysis was performed to detect *Toxoplasma* SAG1and 18SrRNA genes as diagnostic genes. DNA extraction was performed using the DNeasy®Tissue kit (DNG SINACOLON). The primers were designed by BLAST method of NCBI and produced by Pishgam Company.
Forward- and reverse-primers for SAG1 gene were:
5′-GCTGTAACATTGAGCTCCTTGASTTCCTG-3′ and
5′-CCGGAACAGTACTGATTGTTGTCTTGAG-3′
And for 18SrRNA gene was:
cocc18S-F: 5_-GAAAGTTAGGGGCTCGAAGA-3_and

cocc18S-R: 5_-CCCTCTAAGAAGTGATACA-3_

Amplification of the SAG1 and 18SrRNA genes were completed in the following conditions: 1 cycle of 5 min at 95˚C for initial denaturation followed by 30 cycles of 1 min at 95˚C, 1 min at 62˚C, and 3 min at 74˚C. The best annealing temperature was 62◦C. Amplification was performed using a DNA thermal cycler (Eppendorf instrument). PCR amplification products were examined in 1. 5% agarose gels and confirmed by staining with Safe stain and visualized under Gel Doc using UV.

The statistical surveys were done with SPSS18 software.

Results

In this study we found 68 samples of these rodents were positive for SAG1 and 18SrRNA genes. 38 samples were Ratus ratus, 10 samples were Ratus norvegicus, 10 samples were Mus musculus and 10 samples were Rombumys opimus. The samples were positive in 1180bp location for SAG1 gene and 730bp for 18S rRNA gene (Table2, Figure2 & 3).

Rodents Type	Numbers of Samples	Numbers of Positive
Rattus rattus	130	38
Rattus norvegicus	45	10
Mus musculus	60	10
Rombomys opimus	50	10

Table2: The total sample and positive sample for SAG1 and 18SrRNA genes.

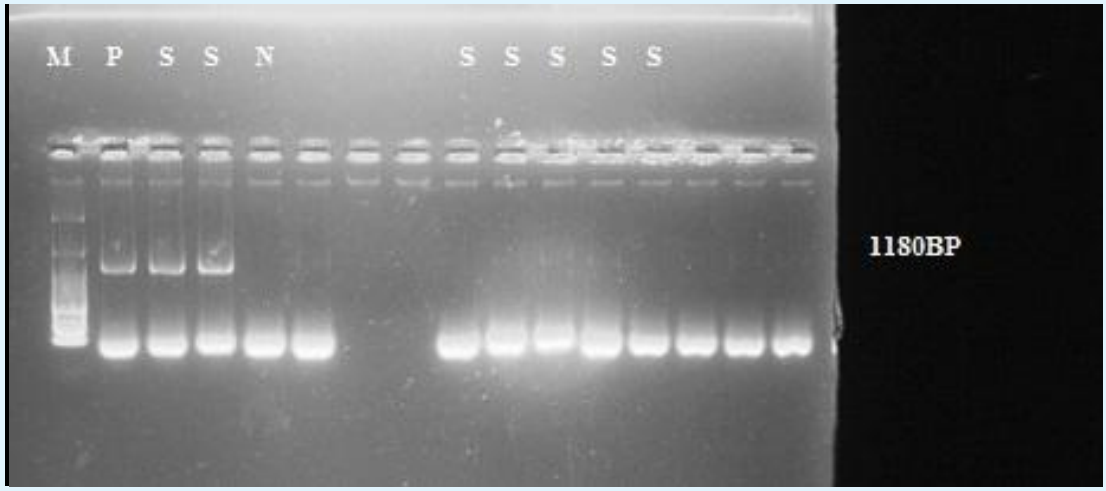

Figure2: PCR result for SAG1gene.
P: positive control, M: size marker, S: positive sample, N: negative control.

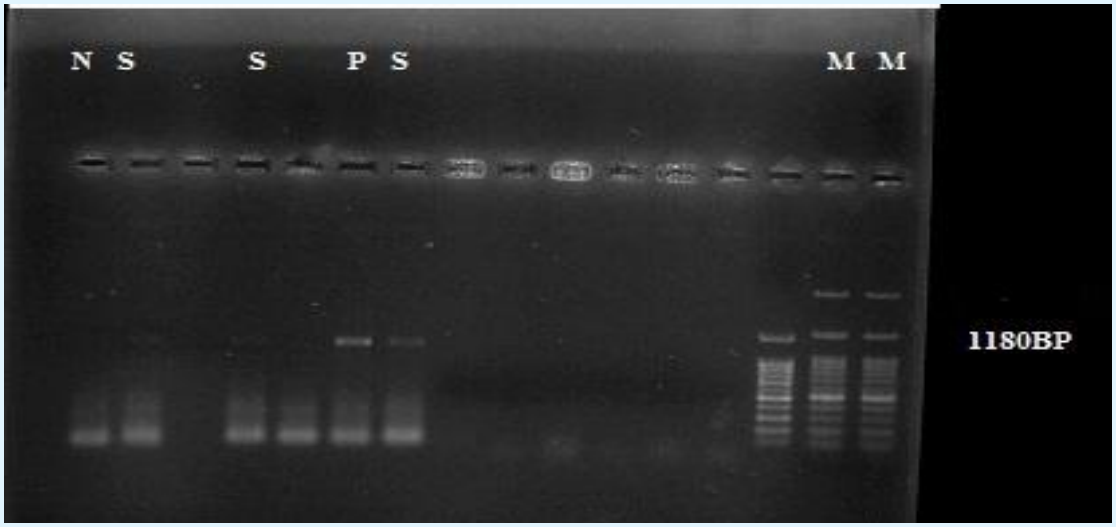

Figure3: PCR result for SAG1gene.
P: positive control, M: size marker, S: positive sample, N: negative control.

These results showed that positive samples in 1180bp area by SAG1 gene. The primer design done by BLAST software from NCBI site in 130 samples from Rattus rattus 38 samples were positive, in 45 samples from Rattus norvegicus 10 samples were positives, in 60 samples from Mus musculus 10 samples were positive and finally in 50 samples from Rombomys opimus 10 samples were positives. The samples were brain and heart tissues from these rodents.

In chart 1 we showed that 1=Rattus rattus, 2=Rattus norvegicus, 3=Mus musculus and 4=Rombomys opimus. Series 1 (blue) were total sample and series 2(red) were positive samples.

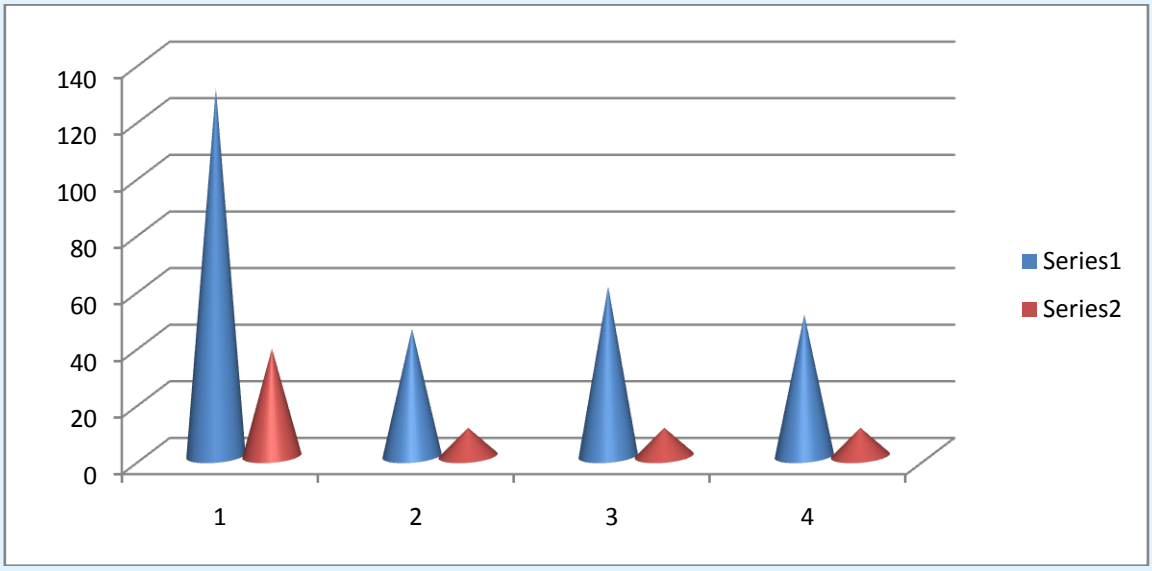

Chart1: numbers of positive.

In Figure 4 we showed that positive samples of rodents for 18SrRNA gene of *toxoplasma gondii* in 730 bp location (Figure 5 & 6).

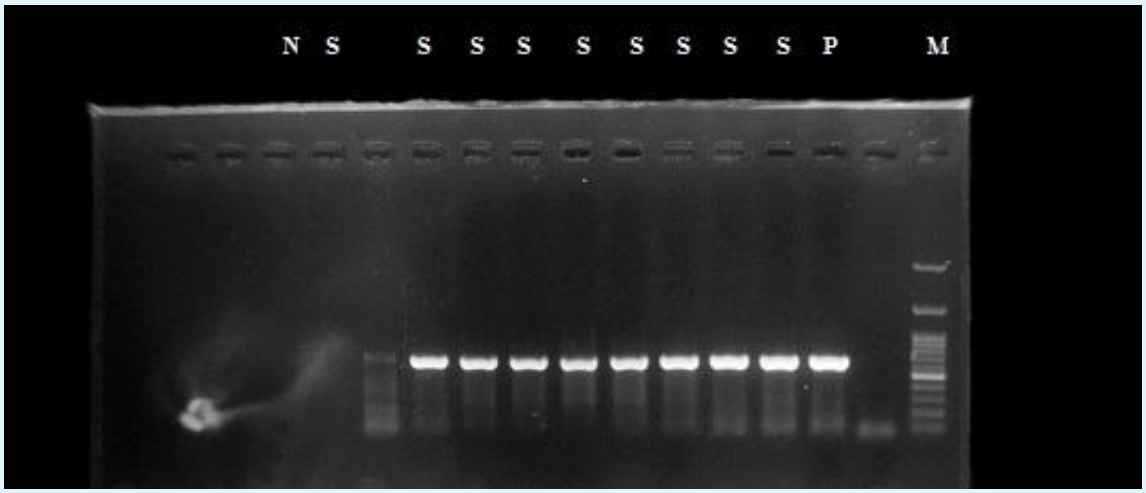

Figure 4: PCR result of *toxoplasmagondii* using 18SrRNA gene. P: positive control. M:size marker. S: sample. N: negative control.

Figure 5*:* File: m24_F. ab1 Run Ended: 2017/3/15 6:32:24 Signal G: 4464 A: 6675 C: 8025 T: 9201
Sample: m24_F Lane: 64 Base spacing: 16. 291637 631 bases in 16116 scans Page 1 of 2. Sequence result of 18SrRNA gene of *toxoplasma gondii.*

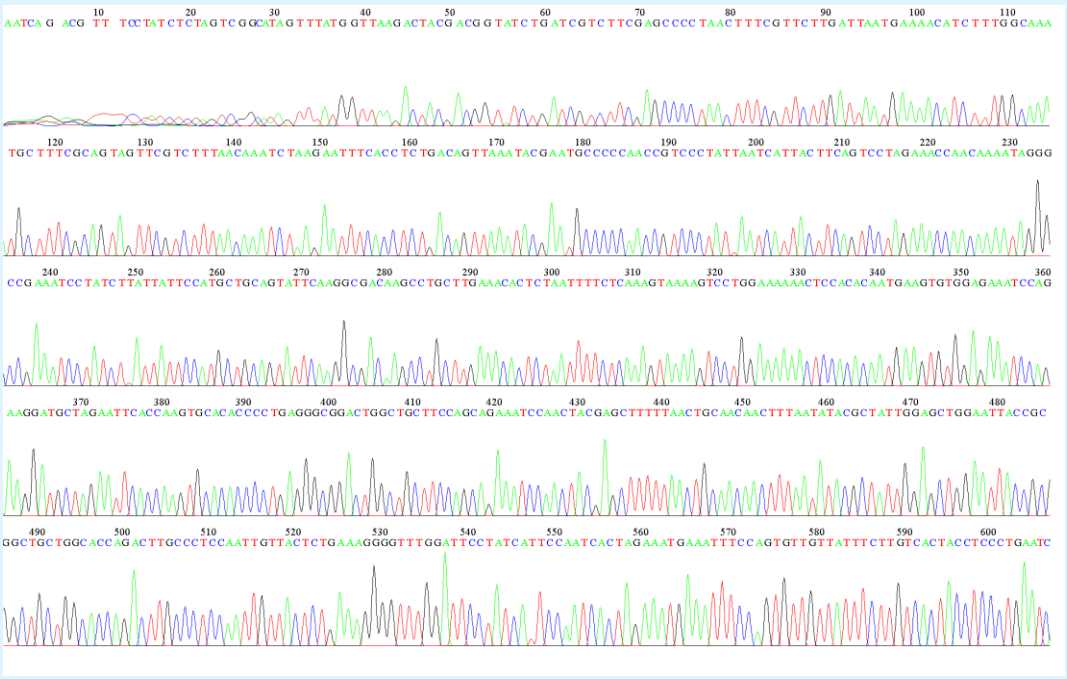

Figure 6: File: m24_R. ab1 Run Ended: 2017/3/15 6:32:24 Signal G: 4657 A: 7548 C: 11547 T: 11255
Sample: m24_R Lane: 62 Base spacing: 15.991175 684 bases in 8365 scans Page 1 of 2. Sequence result of 18SrRNA gene of *toxoplasma gondii*.

Discussion

Regarding to free living of rodents, feral and stray cats and also presence of them in large number in rural areas, obtaining data about *T. gondii* dynamic in rodents and cats' population of rural area is critical for the establishment of monitoring programs [19-21]. My purpose of this study was abundance detection of toxoplasmosis in rodents of golestan province using SAG1 and 18SrRNA genes. Toxoplasmosis is a zoonos is infection between rodents, cats, domestic animals and human. All of them have been introduced toxoplasmosis as one of the major zoonotic infection diseases in Iran. Result of recent study revealed 85% *T. gondii* infection of sampled feral cats from 20 villages of Golestan province. The climate characteristic of Golestan province is very optimum to grow toxoplasmosis infection in that area. Prevalence of 40% *T. gondii* antibodies in stray cats in Sari, Northern Iran, has been reported by Sharif and his colleagues 2009. Sharif, et al. survey anti *T. gondii* antibodies with latex agglutination test (LAT) on 100 serum samples collected from stray cats in five urban areas of Sari. Sari is located near Golestan province in North Iran and has humid climate which has been introduced suitable for *T. gondii* growth. But *T. gondii* infection of stray cats in Sari is lower than *T. gondii* infection of feral cats in Golestan province [22-25]. The significant difference between these two similar studies may be due to difference in sampled population. Result of related studies in Garmsar 82.2, Urumia (86%) and Tehran (89%) had the most similarity with result of current study (85%). A study in Tabriz by Jamali clarified 36/2% *T. gondii* infection of cats by using dye test that differed with our methods. In this study most positive sampled has been belonged to Azadshahr villages and also Gorgan villages. Also Mostafavi, et al. reported highest prevalence of human toxoplasmosis, 70%, in humid regions of North Iran. The sex-seroprevalence pattern found in this study is similar to those found by Raeghi et al. And Haddadzadeh, et al.. Prevalence of toxoplasma gondii antibody in cats in Urmia, North West of Iran. In Sari by contrast, differences in *T. gondii* infection were detected between male stray cats and female stray cats [22-26]. Most of the studies didn't report significant different in *T. gondii* infection of the sexes and the role of sexuality in *T. gondi* exposure is not clear. in recent years has not any studies in rodents toxoplasmosis in golestan area. In 2011 Hong did very important study in genotyping of cat's toxoplasmosis. In 2011 Dubey done a study in genotyping of zoonos is toxoplasmosis in USA. In 2012 Selseleh did a study in genotyping of Tehran rodent's toxoplasmosis by SAG1 gene. In 2012 Habibi had done very important study in detection of sheep toxoplasmosis by SAG1 gene. In 2013 Ling jang had done immense study in detection of rodent toxoplasmosis by SAG1 gene in china. In 2013 Cabral did a study in detection of rodent toxoplasmosis in Brazil. In 2014 Barros, et al. found genetic characteristics of toxoplasma gondii from doves in Brazil. In 2014 Yan c and etc found genetic characteristics of toxoplasma gondii from rodents in china. In 2014 Gjerede, et al. found genetic characteristics of toxoplasma gondii from muscles of Lutra in Norway. In 2014 Chen lj and etc detected toxoplasma gondii from HIV positive people in China [26-30]. These studies and very another surveillances showed important role of rodents and cats in dissemination of toxoplasmosis in humid area. In this study we showed that the SAG1 and 18SrRNA genes were very important markers to detection of abundance of zoonotic toxoplasmosis and brain or heart tissue were main tissues to follow SAG1 and 18SrRNA genes from tachyzoites of toxoplasma parasite, also the rodents are very important reservoirs in dissemination of toxoplasmosis in Golestan area, northeast of IRAN [26-30].

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