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Abundance Detection and Molecular Characterization of *Toxoplasma gondii* by SAG1 Gene in Rodents and Cattle of Golestan Province, Northeast of Iran

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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 and cattle are most reservoir hosts in environment that by attention of human environment vicinity to cattle and rodent's environment causes *Toxoplasma* dispersion in that area. The aim of this study was Molecular detection of *Toxoplasma gondii* in rodents and cattle of Golestan province, northeast of Iran.

Materials and methods: In this study we collected 285 mice and 185 cattle tissues from Golestan forest and extracted brains of rodents and hearts tissues of cattle to obtain DNA 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 gene. 38 samples were *Ratus ratus*, 10 samples were *Ratus norvegicus*, 10 samples were *Mus musculus* and 10 samples were *Rombumys opimus*. Also 81 samples of cattle hearts were positive for SAG1 gene.

Conclusion and discussion: in this study we found that the different types of rodents were responsible to spread of toxoplasmosis, also SAG1 gene was very useful marker to detect toxoplasmosis in rodents of northeast area of Iran. Also in this area the numbers of toxoplasmosis cattle were in very high range.

Keywords: Toxoplasmosis; SAG1 gene; PCR; Golestan forest; Rodents; Cattle

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 disseminating of toxoplasmosis [1-5]. Rodents are very important reservoir in dissemination of toxoplasmosis in Iran. The brain of rodents and the heart of cattle are the most important tissues that can infect by toxoplasmosis. Toxoplasmosis infection causes tissue cysts in these organs of rodents and cattle. The major gene that extracted from these organs to detect toxoplasmosis is SAG1 gene [5-10].

Toxoplasmosis infection pathway is eating of infected tissues from rodents by cats or uncooked meats from domestic animals to human, 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 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 toxoplasmosis infection. My purpose was the abundance detection of toxoplasmosis in rodents and cattle of Golestan province using SAG1 gene 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). 185 cattle were collected from 20 villages of 5 towns locating in different climates of Golestan province. Also rodents were collected from Golestan forest. This study was done on March to September 2016. Because of this study was based on molecular detection; we did not use other examinations for example histopathology examinations.

Preparation of samples

We collected 285 mice from Golestan forest and 185 cattle hearts from 20 villages. Then brains and hearts of mice and cattle were removed. These organs 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*) (Table 1).

DNA extraction

To extraction genomic DNA, we cut approximately 3 g of brain and heart tissues by DNG/proteinase K method from Sinacolon company and eluted into 50 µl DDH2O according to the manufacturer's

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

Table 1: The total samples of rodents divided in 4 groups [14-18].

recommendations. A PCR targeting the *T. gondii* SAG1 gene was performed to detect possible infection with T. gondii. DNA samples giving positive SAG1 amplification were then used for genetic characterization.

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

Added 20 µl proteinase K to the samples. Then mixed immediately by shaking for 20 seconds. Then incubated at 60°C for 1 hour to lyse samples. If tissue was difficult to lyse, we increased the incubation time to 2-3 hours. Then inverted the samples every 10-15 minutes. Then we added 300 microliter isopropanol to DNA precipitation. After 5-10 minutes we washed the tubes by 70% ethanol and finally we eluted the DNA by DDH20. The eluted DNA preserved in -20°C freezer until using PCR method to detection of infection. The purification of DNA was done with Nanodrop instrument.

PCR analysis for *T. gondii* SAG1 gene

To genetically identify the presence of KI-1 Tachyzoites in visceral organs, PCR analysis was performed to detect *Toxoplasma* SAG1 gene as a diagnostic gene. DNA extraction was performed using the DNeasy®Tissue kit (DNG Sinacolon). The primers were designed by BLAST method from NCBI site and produced by Pishgam Company.

Primer sequences were:

5´- GCTGTAACATTGAGCTCCTTGASTTCCTG-3´ for forward and

5´- CCGGAACAGTACTGATTGTTGTCTTGAG-3´ for reverse

Amplification of the SAG1 gene was 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 SPSS 18 software (Table 2).

Results

In this study we found 68 samples of these rodents were positive for SAG1 gene. 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 1180 bp location. Also 81 heart samples of cattle were positive for SAG1 gene. *Toxoplasma gondii* SAG1 gene after NCBI blast, complete cds (Table 3; Figures 1 and 2).

Origin:

1 acaatgtgca cctgtaggaa gctgtagtca ctgctgattc tcactgttct cggcaagggc

61 cgacgaccgg agtacagttt ttgtgggcag agccgttgtg cagctttccg ttgttctcgg

Materials	Volume	Final concentration
Master Mix	7.5 µl	2X
DNA Sample	2 µl	10-100 ng/µl
Work Primer	1 µl	20 pmol
Distilled water	4.5 µl	-
Total	15.0 µl	-

Table 2: PCR substrates [14-18].

Rodents type	Numbers	Numbers of positive
Rattus rattus	130	38
Rattus norvegicus	45	10
Mus musculus	60	10
Rombomys opimus	50	10

Table 3: Total samples and positive samples for SAG1 gene [14-18].

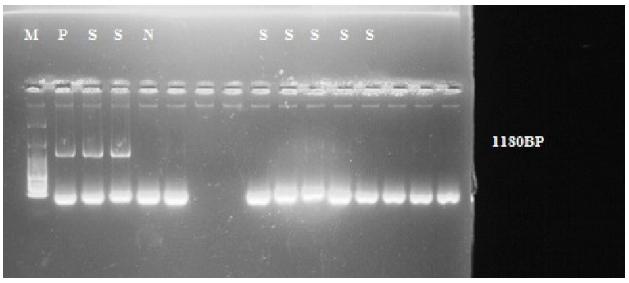

Figure 1: PCR conclusion for SAG1 gene. P: Positive control. M: Size marker. S: Sample. N: Negative control [14-18].

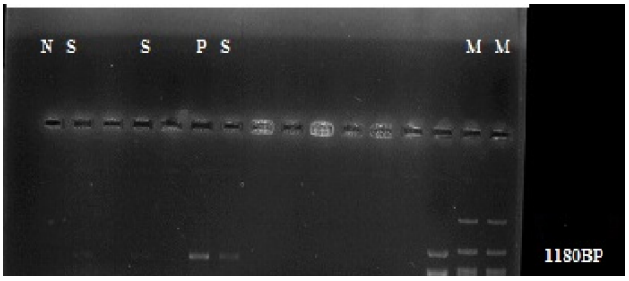

Figure 2: PCR conclusion for SAG1 gene. P: Positive control. M: Size marker. S: Sample. N: Negative control [14-18].

121 ttgtgtcaca tgtgtcattg tcgtgtaaac acacggttgt atgtcggttt cgctgcacca

181 cttcattatt tcttctggtt ttttgacgag tatgtttccg aaggcagtga gacgcgccgt

241 cacggcaggg gtgtttgccg cgcccacact gatgtcgttc ttgcgatgtg gcgttatggc

301 atcggatccc cctcttgttg ccaatcaagt tgtcacctgc ccagataaaa aatcgacagc

361 cgcggtcatt ctcacaccga cggagaacca cttcactctc aagtgcccta aaacagcgct

421 cacagagcct cccactcttg cgtactcacc caacaggcaa atctgcccag cgggtactac

481 aagtagctgt acatcaaagg ctgtaacatt gagctccttg attcctgaag cagaagatag

541 ctggtggacg ggggattctg ctagtctcga cacggcaggc atcaaactca cagttccaat

601 cgagaagttc cccgtgacaa cgcagacgtt tgtggtcggt tgcatcaagg gagacgacgc

661 acagagttgt atggtcacag tgacagtaca agccagagcc tcatcggtcg tcaataatgt

721 cgcaaggtgc tcctacggtg cagacagcac tcttggtcct gtcaagttgt ctgcggaagg

781 acccactaca atgaccctcg tgtgcgggaa agatggagtc aaagttcctc aagacaacaa

841 tcagtactgt tccgggacga cgctgactgg ttgcaacgag aaatcgttca aagatatttt

901 gccaaaatta actgagaacc cgtggcaggg taacgcttcg agtgataagg gtgccacgct

961 aacgatcaag aaggaagcat ttccagccga gtcaaaaagc gtcattattg gatgcacagg

1021 gggatcgcct gagaagcatc actgtaccgt gaaactggag tttgccgggg ctgcagggtc

1081 agcaaaatcg gctgcgggaa cagccagtca cgtttccatt cttgccatgg tgatcggact

1141 tattggctct atcgcagctt gtgtcgcgtg agtgattacc gttgtgc

These results showed that positive samples in 1180 bp location by SAG1 gene. These results obtained from GelDock instrument. Primer designation was 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. Also 81 samples of heart tissues of cattle were positive for toxoplasmosis.

Figure 3 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. Positive samples bands detected on 1180 bp region [14-18]. These results obtained from GelDock instrument (Figure 4). Figure 5 showed that in the 185 heart tissues of cattle, 85 samples were positive for *Toxoplasma* SAG1 gene.

Discussion

Regarding to free living of rodents, cattle and also presence of them in large number in rural areas, obtaining data about *T. gondii* dynamic in rodents and cattle' 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 and cattle of Golestan province using SAG1 gene. Toxoplasmosis is a zoonosis infection between rodents, cats, human and domestic animals. Rodents are main reservoir host for toxoplasmosis to infect feral cats and uncooked meat of domestic animals such as cattle or sheep can transfer toxoplasmosis to human. In both ways the human can plague with toxoplasmosis. In Golestan province, the population of rodents is higher than cattle but both animals have same role in spreading of toxoplasmosis. Climate characteristic of Golestan province is optimum for dissemination of toxoplasmosis. Prevalence of 40% *T. gondii* antibodies in stray cats in Sari, Northern Iran, has been reported by Sharif et al. They also 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 [22-25]. 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. Compare to Bandar-Turkmen,

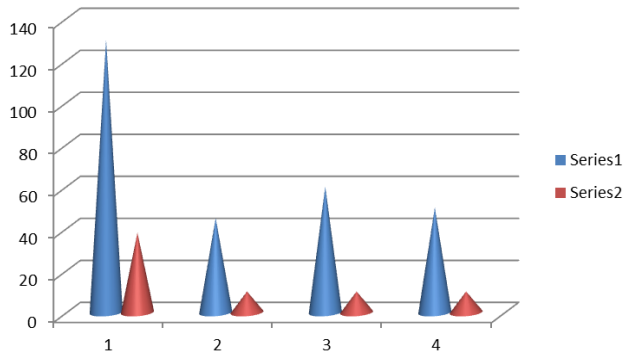

Figure 3: Positive samples from toxoplasmosis rodents [14-18].

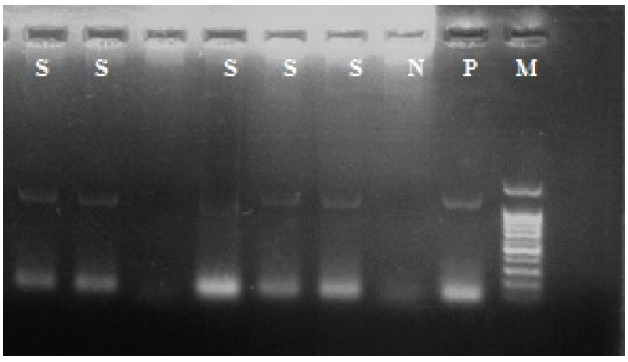

Figure 4: Positive samples of cattle heart tissues. M: Size marker. S: Samples. N: Negative control. P: Positive control.

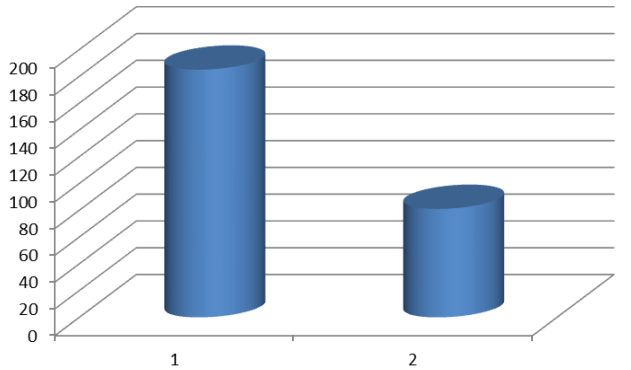

Figure 5: 1=Total heart samples of cattle. 2=Number of positives for toxoplasmosis cattle [14-18].

Gonbad and Aghghala that have semi-arid and arid climates, these two regions have humid climate. So detection of higher positive sample in Azadshahr villages and also Gorgan villages can be due to more suitable condition for growth of *T. gondii* oocyst in these areas. Also Mostafavi et al. reported highest prevalence of human toxoplasmosis, 70%, in humid regions of North Iran. Prevalence of *Toxoplasma gondii* antibodies in cats from Urmia, Northwest of Iran. In Sari by contrast, differences in *T. gondii* infection were detected between male stray cats and female stray cats. In 2013, Cong detected house sparrows toxoplasmosis in China, Khademvatan detected birds toxoplasmosis in southwest of Iran and Ortega found pigs toxoplasmosis in Mexico [22-26]. Most of the

studies didn't reported significant different in *T. gondii* infection of two sexes and the role of sexuality in *T. gondii* exposure is not clear. In recent years had 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 zoonosis toxoplasmosis in USA. In 2012, Selseleh did a study in genotyping of Tehran rodent's toxoplasmosis by SAG1 gene. In 2012, Habibi did very important study in detection of sheep toxoplasmosis by SAG1 gene. In 2013, Ling jang did 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. had found genetic characteristics of *Toxoplasma gondii* from doves in Brazil. In 2014, Yan et al. found genetic characteristics of *Toxoplasma gondii* from rodents in china. In 2014, Gjerede et al. had found genetic characteristics of *Toxoplasma gondii* from muscles of Lutra in Norway. In 2014, Chen et al. detected *Toxoplasma gondii* from HIV positive people in China [26-30]. In recent years have not perform any studies to detect rodents and cattle toxoplasmosis at the same time in Golestan area. This study showed important role of rodents and cattle in dissemination of toxoplasmosis in humid area. In this study we showed that the SAG1 gene was very important marker to detection of abundance of zoonotic toxoplasmosis and brain or heart tissue was main tissues to follow SAG1 gene from Tachyzoites of *Toxoplasma* parasite, also the rodents and cattle were very important reservoirs in dissemination of toxoplasmosis to human in Golestan area, northeast of Iran [26-30].

Conclusion

In this study we concluded: The northeast of Iran was very important region for following toxoplasmosis infection, specifically in animals such as rodents and cattle. In other study that had done four month after this surveillance, we found that the dominant genotype of *Toxoplasma gondii* in that area was genotype using PCR-RFLP by HaeII restriction enzyme.

Acknowledgements

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