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Genotyping of *Toxoplasma gondii* in Sheep and Cattle Meat Using PCR-RFLP Technique

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ABSTRACT

Toxoplasma gondii is an obligate, intracellular parasite, with worldwide distribution. The main source of infection for humans is livestock and meat-producer animals. The relationships between *Toxoplasma* genotype and biological characteristics of the parasite have already been identified. According to the pathogenicity of the parasite in laboratory animals, *Toxoplasma* is divided into three genotypes included type I, II and III. Understanding the genotype of the parasite, could help us to predict clinical features and severity of disease. The aim of this study was to identify genotypes of *T. gondii* in cattle and sheep meat and meat products in Ahvaz city southwest of Iran. One hundred and ninety samples of tongue, heart and muscles of sheep and cattle and meat products, including sausages and burgers, were collected from slaughterhouses and stores. To identify *Toxoplasma gondii*, DNA were extracted from samples and B1 gene were amplified by specific primers. To determine the genotype of *T. gondii*, PCR-RFLP was done on positive samples using by amplifying GRA6 gene and endonuclease MseI enzyme. Data analysis showed that the strain of the parasite in all positive samples belonged to genotype I. In this study the predominant *Toxoplasma* genotype was type I which can cause severe clinical symptoms in immunocompromised patients. Further research is needed to determine the genotype of the parasite in humans and other animals.

1. Introduction

T*oxoplasma gondii* is an obligate intracellular protozoan parasite with global distribution^[1]. The parasite is belonged to coccidian parasites and cause infection in humans and other warm-blooded mammals^[2]. The Parasite is transmitted to humans mainly by contaminated drinking water or ingestion of raw or undercooked infected meat^[3].

The prevalence of infection in the world, depends on several factors such as location, weather and culture^[4]. It is estimated that more than a third of the world's population are infected with *Toxoplasma*. In the US, 20 to 30 percent of the human population are seropositive. Other reports were recorded in Japan 25%, Netherlands 60%, Italy 60%, Finland 35%, France 50%, and Poland 50%-60%^[5]. It is well known that meat has important role to cause human toxoplasmosis infection^[2]. The seroepidemiology studies

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against *Toxoplasma* infection on slaughtered animals and birds show high contamination with of 26.6% to 88% in studied animals [6,7]. The meat products like sausages and burgers were also infected with *Toxoplasma* cysts [8].

There are three genotypes of the parasite in the domestic cycle which includes type I, type II, and type III [9,10]. In the human population, type II parasites are predominant and cause congenital toxoplasmosis in Europe but type I are more frequently associated with severe forms of toxoplasmosis in immunocompromised patients and acquired ocular toxoplasmosis [11]. Recognition of parasite genotypes helps us to understand biology, epidemiology and heterogeneity pattern to develop a new strategy in treatment and prevention. Regarding to the lack of the information about the parasite genotype in Ahvaz southwest Iran, this study has been done using PCR-RFLP technique.

2. Material and Methods

2.1 Samples

One hundred samples of sheep and cattle (each 50 sample) of tongue, heart and muscle were collected from slaughterhouses and stores. In addition, 90 samples of burgers, sausages and kielbasa (each 30 samples) were prepared from the stores. To prevent cross contamination, knife after each sample was rinsed. The tissue samples were stored at -20°C until used.

2.2 PCR and Genotyping of *T. gondii*

B1 gene was selected for PCR because of highly conservation among *Toxoplasma* strains with 35-fold repetition and 2214 nucleotides in each repeat. This gene was targeted for PCR by using specific primer Tg1, Tg2 and amplification of a 469-bp DNA fragment [9]. Forward primer was, 5'AAAAATGTGGGAATGAAAGAG-3' and reverse primer was, 5'ACGAATCAACGGAAGTGAAT-3'. Each reaction was carried out in a final volume of 50 µL containing 1.5 µL MgCl₂, 1 µL of each primer, 1 µL of each deoxynucleotide triphosphate, 5 µL PCR buffer, 20 µL of DNA template, 0.3U Taq DNA polymerase and 20.2 µL Distilled water. The PCR was done according to following conditions: one cycle of initial denaturation 5 min at 95 °C followed by 35 cycles of 94 °C for 30 s, 60 °C for 1 min, 72 °C for 2 min, and was ended by 1 cycle of final extension at 72 °C for 7 min (Bio Rad-USA thermal cycler). The PCR products were run in 1% agarose gels with staining by ethidium bromide. The Bands were visualized under UV light (Uvitec-Iran).

2.3 PCR-RFLP

The GRA6 gene which is appreciated for RFLP-PCR

was selected. The primers included: GRA6F:5'GTAGC-GTGCTTGTGGCGAC-3' and GRA6R: 5'TACAAGACATAGAGTGCCCC-3'.

The PCR program was:

First denaturation 5 Min at 95 °C and followed by 35 cycles contained denaturation 30 second at 94 °C, annealing 60 second at 60 °C, extension 2 min at 72 °C and one cycle for final extension 7 min at 72 °C.

Thirty-five microlitres of PCR product was digested using 0.3 µL of MseI endonuclease enzyme (Vivantis-Malaysia) and incubate at 65 °C for 1h. The restriction fragments were separated by electrophoresis in 2% agarose gel and was visualized under UV.

3. Results

According to finding of current study, 9 out of 190 (4.7%) samples [sheep, cattle and meat products] were positive for *Toxoplasma gondii* by PCR. The highest infection was seen in sheep samples with 7 out of 50 (14%) samples and the least infection was seen in cattle with 2 out of 50 samples (4%). The B1 gene was amplified and a 469 bands was shown in agarose (Figure 1). SevenAll positive samples in primary PCR were selected for RFLP-PCR. A of 800 base pair band was seen in second PCR. According to the restriction map information, all samples were belonged to type I (Figure 2). After the enzymatic digestion, 800 bp fragment has been divided into two fragments: approximately 600 and 200 bp fragments (Figure 2).

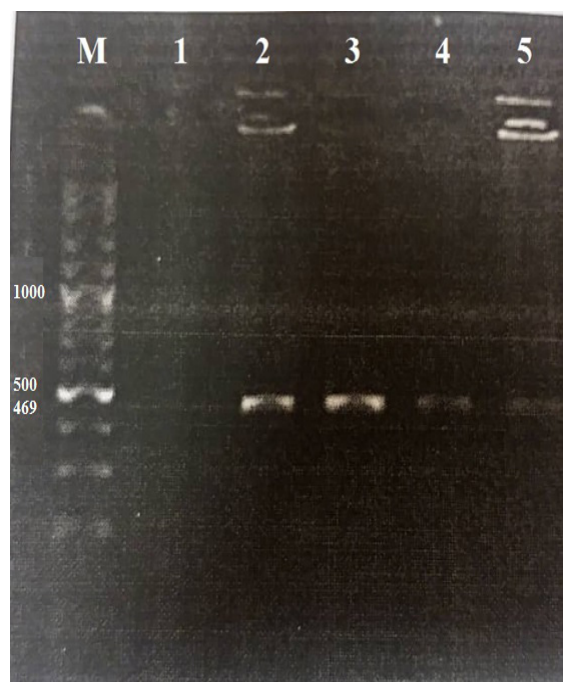


Figure 1. PCR of B1 gene in samples show a 469 bp band

Note: M: Marker, Line1 Negative control, Line 2,3,4,5 positive samples.

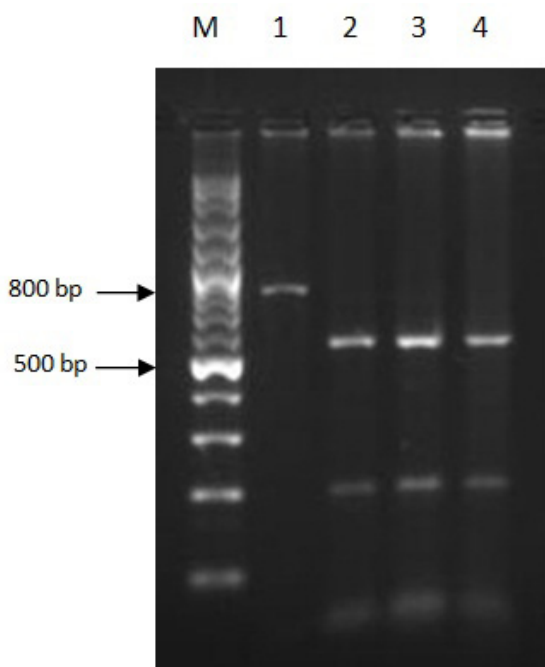


Figure 2. PCR-RFLP analysis of GRA6 with MseI endonuclease

Note: Lane M is marker (100bp), 1 Positive samples before enzymatic digestion, fragment sizes 800 bp, Lane 2-4 are *T. gondii*, type I.

4. Discussion

Toxoplasma infects a variety of warm-blood animals including mammals and birds [10]. In our study the parasite was not isolated from any sample of meat products (burgers, sausages and kielbasa), which may be due to the low number of samples or low contamination level in meat products. It is likely that the *Toxoplasma* transmission by meat production are less important in this area but 14% of sheep and 4% of cattle are infected. Warnekulauriya *et al* showed one sample out of 67 meat samples including dried and semi-dried sausages and hams has been positive for *Toxoplasma* by using PCR technique. They believed that to infect humans, this percentage of contamination in meat is sufficient [11]. It is difficult to find *T. gondii* tissue cysts in large animal because of the less chance of detection of the parasite in cattle compare to sheep and more resistance of cattle against infection. Dubey has estimated that less than 1 tissue cyst/50 g of tissue is detectable in pigs [12]. Thus, false-negatives can result from insufficient sample size. The relationship between parasite genotype and clinical feature and on the other hand, biological characteristics has been interested for many years ago. Zhang *et al* revealed that different genotype of *T. gondii* may have inherent ability to stimulate different host immune system which lead to various clinical symptoms and severity of

the disease [13]. The relationship between *Toxoplasma gondii* and Alzheimer's disease (AD) was interested in recent years. It seems that *T. gondii* as a neuroleptic intracellular parasite cause injury in neuron and increase expression of phosphorylated tau (p-tau) in the hippocampal tissue which lead to AD in experimental toxoplasmosis. Also Tao *et al* presented genotype I of *T. gondii* can cause apoptosis in neuron cells by increasing expression of caspase3. They concluded that neurofibrillary pathologic changes can predispose to formation of Alzheimer's disease in brain toxoplasmosis [14].

In this study, for identification genotype of the parasite, PCR-RFLP method using GRA6 gene was applied and two fragments included 600 and 200 bp were detected. The predominant genotype which isolated from sheep and cattle meat samples was type I. This finding is in agreement by Falah *et al* study [15]. They also reported the genotype I in the meat products [15]. Zia-Ali *et al* isolated genotypes II and the III in birds in Mazandaran north of Iran [16]. Aspinal *et al* showed 85% of samples with SAG2 gene belonged to type I [17]. Different genotypes of the parasite may be reported according to different geographical areas. In United State, from seven positive meat samples, three samples genotype III, 2 samples genotype II and 2 samples genotype I has been reported [18]. Schumacher *et al* showed 9 out of 12 person who consumed undercooked meat of venison in Wisconsin USA were infected by *Toxoplasma gondii* type V and clinical symptoms included fever, chills, sweats, and headache (100%) and ocular disturbances (33%). One person did not clinical symptoms because of having antibody against *Toxoplasma* from previous infection [19].

In Switzerland, predominant genotypes in cattle were type I and III [20]. In Brazil a study on swine sausage indicated that 73.68% and 26.32% of the parasite genotype belonged to I and III respectively [21]. Ivovic *et al* presented 2.94% out of 136 trapped rodents were infected by *T. gondii* genotype II using RFLP-PCR [22]. Oliveira isolated *T. gondii* type II from an outbreak of abortion in goats in Brazil [23]. Silva *et al* presented five genotype of sheep in Rio Grande Brazil including type II (TgOvBrRS4), type BrIV (TgOvBrRS2 and TgOvBrRS3) and two new non-archetypal genotypes [24]. In Japan 44.8 %, 48.3 % and the 6.9% were reported type I, II and the III, respectively [25]. These results indicate that, there are difference parasite genotype according to geographic area and host. There are many genotype of *T. gondii* in the world and different symptoms and a range of disease severity are dependent to parasite genotype. In this study predominant genotype of *Toxoplasma gondii* in ruminant was type I. This type of parasite induces several clinical sign especially in immu-

nocompromised patients. Furthermore study for precise determination of genotype of *Toxoplasma* on other mammals and birds is needed and the clinical signs should be compared with parasite genotype.

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Conflict of Interest

There is no any conflict of interest.

Authors' Contributions

Mahmoud Rahdar designed the original idea and collected the samples and writing the paper. Alireza Samarbaf-zadeh consulate for molecular technique and participate to writing proposal and analyzed results. Laila Arab participate for writing proposal and primary drafting of paper and doing practical molecular technique. All authors reviewed and confirmed the final paper.

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