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Development of PCR assay for detection of *Toxoplasma gondii* in meat products in south west of Iran

Rahmatollah Yazdani, Abbas Doosti*, Marzieh Shavali, Asghar Arshi

Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

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Abstract

Toxoplasma gondii (*T. gondii*) is an intracellular protozoan parasite with felids as definitive hosts, including humans and domestic animals and is an important cause of abortions and stillbirths. The aim of present study was to identification of *T. gondii* in meat products by PCR assay in Chaharmahal Va Bakhtiari province (south west of Iran). In the present study, 273 meat products were collected from different companies and food markets. Genomic DNA was extracted and PCR was performed for *B1* gene amplification of meat products using specific oligonucleotid primers (Tg1 and Tg2). Results showed that 30 out of 273 samples (10.98 %) were positive for *T. gondii*. These findings showed relatively high incidence of *T. gondii* infection in meat products in south west of Iran.

* **Corresponding Author:** Abbas Doosti ✉ geneticsshki@yahoo.com

Introduction

Toxoplasma gondii (*T. gondii*) is a protozoa, obligate, intracellular parasite with world repartition. It is able of infecting all warm-blooded animals and nearly 30% of the human society (Aspinall *et al.*, 2002; Sibley *et al.*, 2009). This parasite has been classification in three genetic types (I–III) based on restriction fragment length polymorphism (RFLP) (Fallah *et al.*, 2011; Dubey *et al.*, 2006). The overall, *T. gondii* infections are no signs of and self-limiting, particularly among healthy immunocompetent hosts; however, the infection may cause severe complications in pregnant women and immunocompromised patients (Kaul *et al.*, 2004). In their life cycle, conclusive and wild felines are definitive hosts whereas human, other mammals and birds are its interface hosts (Zakaria, 2011; Tilahun *et al.*, 2013). Major ways of infection are: I. Swallowing of oocysts through close contact with infected cat or cat's faeces, II. Eating water or food infected the oocysts, III. Eating crude or undercooked meat from infected animals that contain the tissue cysts, IV. Relocation of infected organs, and V. Congenital infection (Ertug *et al.*, 2005; Kijlstra *et al.*, 2004). Expenditure of undercooked meat is one of the most important ways (28 %) of transfer between pregnant toxoplasmosis patients and has been considered as the most important risk factor of primary infection during pregnancy (Rahdar *et al.*, 2012; Kapperud *et al.*, 1995). Studies have found evidence of extensive *T. gondii* infection in meat-producing animals, particularly cow, sheep and goats. Moreover, such products often contain meat from multiple animals in a single serving. Together these factors result in a higher potential risk of infection after consumption unless these foodstuffs are very well cooked (Tenter *et al.*, 2000; Dubey, 2000). *T. gondii* rampancy in Iran is told to be up to 50 % which increases from dry to humid provinces in north of Iran. The evidence suggesting that up to 63 % of seroconversion during pregnancy happens after undercooked or raw meat consumption (Rahdar *et al.*, 2012). Serological review indicated that toxoplasma infection exists largely among sheep, and goats used for meat production. The *B1*, *P30*, and *ribosomal* DNA genes are highly conserved in all *T. gondii* strains tested to date, and the *B1* and *ribosomal* genes are multiple copy genes within the *T. gondii* genome, making them ideal targets for PCR amplification. The *B1* gene is 35-fold and 2214 nucleotides in each repeat with unknown function. Within eukaryotes, *ribosomal* DNA is frequently repeated, and within *T. gondii* there are over 100 highly conserved copies within the genome (Jones *et al.*, 2000).

This study was conducted to determine *T.gondii* prevalence in slaughtered meat products in Charmahal Va Bakhtiari province (south west of Iran) using molecular methods.

Materials and methods

Sample Collection

A total of 273 samples including 45 salami, 70 sausage, 80 hamburger, 30 hams and 48 frankfurters samples were collected from different factories from Charmahal Va Bakhtiari province. Samples were kept in freezer at -12° C before been used.

DNA Extraction

Genomic DNA was extracted using DNA isolation kit (CinnaGen Co, Iran) according to manufacturer's instruction. The gel monitoring was used for determination of the DNA quality and quantity. All DNA extracts were stored at -20°C until they were used.

PCR Amplification

B1 gene was targeted to procreate specific primers Tg1, Tg2 and amplified 469 bp DNA fragment of the B1 gene with the following primers: Tg1 5'AAAAATGTGGGAATGAAAGAG 3' and Tg2 5'ACGAATCAACGGAACTGTAAT 3'. The PCR reaction was performed in a total volume of 25 µL containing 1 µg of genomic DNA, 1 µM of each primers (Tg1 and Tg2), 2 mM Mgcl2, 200 µM dNTP, 2.5 µL of 10X PCR Gold buffer and 1 unit of *Taq* DNA polymerase (CinnaGen Co, Iran). This solution was initially denatured at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 52°C for 30 s, 72°C for 1 min, with a final extension step at 72°C for 7 min in a

thermal cycler (Mastercycler gradient, Eppendrof,Germany).

Analysis of PCR Products

10 µl of amplified products were run in 1% agarose gel electrophoresis. The electrode buffer was TBE (Tris-base 10.8 g 89 mM, Boric acid 5.5 g 2 mM, EDTA (PH 8.0) 4 ml of 0.5 M EDTA (PH 8.0), combine all component in sufficient H_2O and stir to dissolve). Gels were stained with ethidium bromide. Aliquots of 10 µl of PCR products were applied to the gel. Two control samples were used for each PCR cycle including *T. gondii* DNA as positive and distilled water as negative control. Constant voltage of 80 V for 30 min was used for products separation. After electrophoresis, images were obtained in UVI doc gel documentation systems (UK).

Statistical analysis

All data were analyzed by using MS Excel 2007 and SPSS software (Version 17.SPSS Inc, USA) and p value was calculated using Chi-square and Fisher's exact tests to find any significant relationship. P value less than 0.05 was considered statistically significant

Results

The results showed that 6 of 45 salami (13.3%), 6 of 70 sausage (8.57%), 11 of 80 hamburger (13.75%), 3 of 30 hams (10%) and 4 of 48 frankfurters (8.33%) samples were found to be contaminated with *T. gondii* (Table 1). These results showed that total positivity rate was 10.98 % in 273 samples. The highest and lowest rate were for Hamburger and Frankfurters (13.75 and 8.33) respectively. The results of PCR were shown in Figure 1.

Table 1. *T. gondii* samples detected in meat products from Charmahal Va Bakhtiari province.

Meat product	Number of samples	positive samples	Percentage
Salami	45	6	13.3
Sausage	70	6	8.57
Hamburger	80	11	13.75
Hams	30	3	10
Frankfurters	48	4	8.33
Total	273	30	10.98

Discussion

Toxoplasmosis is one of the maximum common human infections all over the world; infection is more common in highland regions and lower height in cold climates and the hot weather. Widespread dissemination of toxoplasmosis has been shown among animals which are considered for meat production; so unfeasible meats are a serious risk factor in transfer of toxoplasmosis (Ergyn *et al.*, 2009). Sporulated *T. gondii* oocysts stay infective in wet soil for more than 18 months and very insistent to environmental conditions. Anyway, they do not survive lengthy under dry and cold conditions (Dubey, 2000). There are two basic shape of toxoplasma organism: I) the oocyst, which is pour in the cat stool, II) the toxoplasma tissue phase, which found in sheep and cattle (Khadi *et al.*, 2009). When the infected person is a pregnant woman, the toxoplasma organism may blow over into the placenta, the amount of damage done to the mother and the fetus baby depends on at the time of infection the stage of pregnancy (Remington and Desmonts, 1990). Infection in early pregnancy may result in stillbirth or abortion or in a child with various severe neurological conditions including microcephaly, hydrocephalus, and retardation and varying degrees of blindness (Mead *et al.*, 1999). The infected uncooked pork was believed to be a main meat source of *T. gondii* infection in the world for humans (Joan, 2005).

In the studies in 2006, 2008, 2009 and 2012 were done in Iran, The frequency of *T.gondii* in meat products in the provinces of the following results were obtained 30%, 9% and 35% in goats, cattle, and sheep respectively in Mazandaran province (Sharif *et al.*,

2006). In another study the positive rates of sheep and goats were 24.7% and 15.8% respectively in Kerman (Bahrieni *et al.*, 2008). Anti-Toxoplasma antibodies were observed in sera of 34.9% animals in Shiraz city. Also the highest rate of infection were found in the cow 55%, and then dogs, horses, sheep, goats and turkeys 51.5%, 40%, 29.5%, 18.8% and 11.1% respectively. Of course no antibody was detected in any of geese (Asgari *et al.*, 2013). Rahdar *et al.* stated at total 4% of beef and 14 % of lamb were found as positive for *T. gondii* in Ahvaz city. Total positivity rate was 4.7% samples (Rahdar *et al.*, 2012). The serological rate of toxoplasmosis in sheep in Gilan and Mazandaran in North of Iran and Khuzestan in southwest of Iran by Ghorbani *et al.* have reported 29-31 %, 32.5-35.8 % and 12.6 % respectively (Ghorbani *et al.*, 1983).

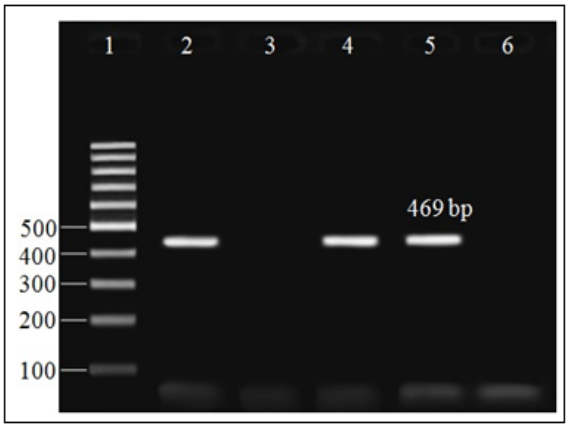

Fig. 1. Gel electrophoresis for detection of *T.gondii* infection in meat products. Lane 1 is 100 bp DNA ladder (Fermentas, Germany); lane 2, 4 and 5 are positive samples, lane 3 and 6 are negative samples.

Results obtained show between women of childbearing age *T.gondii* seroprevalence of 15.0% and an altogether age-appropriate seroprevalence of 22.5%. These results demonstrate that the United States of America is low *T. gondii* seroprevalence in analogy with other countries similar many nations in Latin America and sub-Saharan Africa or France (Jones *et al.*, 2001). A prior study per Warnekulasuriya *et al.* in 1998 detected one positive in a total meat samples, 1.5% contamination (Aspinall *et al.*, 2002). In the present study, we confirmed existence of *T. gondii* in meat products. So the infection risk in meat-products significant importance as to the transmission of *T. gondii* to humans. The rate of *Toxoplasma* infection in meat-products in the current study was relatively high. It is significant that the majority of animals are raised by nomadic clan. In Charmahal Va Bakhtiari province, nomads and their animals move from one place to other place within province or vicinage provinces during winter and summer in search of food for their animals, in conclusion this may increase the chance of obtain the infection while their animals are grazing in different regions with relatively different climates. According to the obtained results, it is suggested that not only immune compromised patients and pregnant women should be addressed but also the whole population should be aware on how to prevent *Toxoplasma* infection.

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