Prevalence of *Toxoplasma Gondii* in Food products in North West of Iran in 2010

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**Abstract:** *Toxoplasma gondii* is an intracellular obligate parasite that causes toxoplasmosis in warm-blooded animals including humans. The transmission of the disease is usually attributed to ingestion of undercooked or raw meat products. The aim of this study was to detection of *Toxoplasma gondii* in meat products using molecular method in East Azerbaijan–North West of Iran. DNA was extracted from 164 meat product samples obtained from 15 commercial establishments. Nested-polymerase chain reaction with primers specific for *Toxoplasma gondii* SAG2 locus was used for detection of the parasite in samples. *Toxoplasma gondii* DNA was detected in 25%, 20.3%, 21.8%, and 32.8%, in salami, sausage, hamburger, kebab samples, respectively. The results of this study indicated high prevalence of *Toxoplasma* contamination in food products in study region

**Key words:** *Toxoplasma gondii*, SAG2, Meat Products, Iran.

**INTRODUCTION**

*Toxoplasma gondii* infections are widely prevalent in human beings and animals with a world-wide distribution (Dubey and Beattie, 1998). Infection with the parasite occurs via ingesting tissue cysts from undercooked meat, consuming food contaminated with oocysts or by accidentally ingesting Oocysts from the environment (Dubey et al., 2000). However, only a small percentage of exposed adult humans develop clinical signs. The severity of *T. gondii* infection in humans depends on different factors (Sibley and Howe, 1996), including host factors (immunity and genetic background) and parasite factors (strain, inoculum size, parasite life-cycle stage).This parasite has been classified into three genetic Types (I–III) based on restriction fragment length polymorphism (RFLP) (Howe et al., 1997; Da Silva et al., 2005; Howe and Sibley, 1995). Meat-producing animals can be infected with *T. gondii* and considered as the most important source of *T. gondii* (Tenter et al., 2000).Older animals that have a higher prevalence of this organism, are usually used in the production of sausages, salami and cured meats (Dubey, 2000) additionally, these products often contain meat from different animals in a single serving so foodstuffs should be completely cooked. Despite a high prevalence of *T. gondii* in Iran (Assmar et al., 1997; Sedaghat et al., 1978; Ghorbani and Samii, 1973; Hashemi-Fesharki, 1996), there are few reports of *Toxoplasma* isolates from meat products in this country. This study was conducted to evaluate *Toxoplasma gondii* infection in meat products in Iran.

**MATERIALS AND METHODS**

**Sample Collection:**
A total of 164 samples including 48 Salami, 46 Sausage, 40 Hamburger and 30 Kebab samples were collected from different factories from East - Azarbayjan province. Samples were kept in the freezer (-12ºC) before being used.

**DNA Extraction from Tissue Samples:**
DNA was extracted from tissue sample (50mg each) using AccuPrep® Genomic DNA Extraction Kit (Bio Neer) according to manufacture’s instruction. All DNA extracts were stored at -20ºC until use.
Amplification of SAG2 locus for Detection of Toxoplasma gondii by PCR:

In order to detect *Toxoplasma gondii*, we used the Polymerase Chain Reaction (PCR) method with following protocol. The reagent mixture consisted of sterile distilled H2O (16.75 μl), dNTPs (0.2 μM), 10X PCR buffer (2.5 μl), MgCl2 (1.5mM), primers (10 pM each of Forward and Reverse), Taq DNA polymerase (1 unite) and template DNA (200 ng). DNA amplification was performed with initial denaturation at 94°C for 4 min, followed by 38 cycles of amplification for PCR and 30 cycles for Nested-PCR (denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 30 sec), ending with a final extension at 72°C for 5 min. Positive results were revealed by electrophoresis on a 1.2% agarose gel at 100V in 1X TAE (Tris Acetate EDTA) buffer. For Amplification of SAG2 gene, Specific primers were used (Eurofins MWG Operon, Germany) and PCR amplification of SAG2 gene was performed as described by Duby (1988). Briefly, the extracted DNA was used as template for first PCR reaction to amplify the region of 5' and 3' flanking sequences of *Toxoplasma gondii* surface antigen P22. In the second set of reactions, the product of the first reaction was employed as template to amplify a fragment corresponding to internal fragments of 5' and 3' flanking region of antigen P22 antigen (SAG2 gene), respectively (Khodai *et al.*, 1988; Burg *et al.*, 1989). *T. gondii* positive control (genomic DNA from strain RH) was included in all PCR experiments to ensure that the PCR was functioning correctly, and a water template as a negative control was used. The primer sequences, annealing sites and sizes of the PCR products are listed in Table 1.

**Table 1:** Names and sequences of the polymerase chain reaction primer pairs used.

<table>
<thead>
<tr>
<th>Markers</th>
<th>External primers (PCR)</th>
<th>Location on the SAG2 gene</th>
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<tbody>
<tr>
<td>5' SAG2 primary PCR</td>
<td>F1: CTCGAACAGGAAACACAAAAGG</td>
<td>217-236</td>
</tr>
<tr>
<td></td>
<td>R1: AGGGGTGCCCTCAACAGTCTTC</td>
<td>3347-3366</td>
</tr>
<tr>
<td>3' SAG2 primary PCR</td>
<td>F3: TCTGTTCCTCGGGAAGTTGACTTC</td>
<td>2165-2185</td>
</tr>
<tr>
<td></td>
<td>R3: GGTATTTCAAAGGCGTGCAATTTC</td>
<td>2761-2781</td>
</tr>
<tr>
<td>5' SAG2 secondary PCR</td>
<td>F2: GAAATGTTTCAGGTTGCTG</td>
<td>197-215</td>
</tr>
<tr>
<td></td>
<td>R2: AAGAGCGAACATTGAAACACAAAC</td>
<td>416-436</td>
</tr>
<tr>
<td>3' SAG2 secondary PCR</td>
<td>F4: ATTCCTCATGGCTCGGTTC</td>
<td>1171-1189</td>
</tr>
<tr>
<td></td>
<td>R4: AACGTTTTCAGGAGGCACAC</td>
<td>1373-1392</td>
</tr>
</tbody>
</table>

**Fig. 1:** Nested-PCR amplification of *T. gondii* SAG2 locus. Lanes1-7 in the left and right of the size marker represent the 5’ and 3’ ends of SAG2 gene that were amplified by Nested-PCR, respectively. Lane 6 corresponds to the RH strain as a positive control, lane 7 corresponds to negative controls and lane 4 contain positive sample.

**RESULTS AND DISCUSSION**

**Identification of T. Gondii Positive Meat-products Samples:**

DNA was extracted from 164 meat samples, consisting 48 Salami, 46 Sausage, 40 Hamburger and 30 Kebab samples and screened for *T. gondii* DNA. In this study out of 164 samples, amplification of 5’ flanking region was successful in 52 samples and 3’ flanking amplification was positive in 65 cases. 45 samples were positive and 100(61%) samples were negative for both flanking regions, respectively. Samples that found to be positive for either 5 or 3 SAG2 PCR were classified as positive for *T. gondii* contamination. In this way, 64 (39%) of the 164 meat samples were found to be contaminated with *T. gondii*, including 16(25%) of the
salami samples, 13(20.3%) of the sausage sample, 14(21.8%) of the hamburger and 21(32.8%) of the kebab samples.

Discussion:

One-third of the human world population is infected with the protozoan parasite *Toxoplasma gondii* (Dubey and Beattie, 1998). High prevalence of toxoplasmosis has been shown among animals which raised for meat production; therefore undercooked meats are an important risk factor for transmission of toxoplasmosis (Ergin *et al*., 2009). This is the first report of the detection of *T. gondii* by PCR technique in meat and meat products in Iran. Since this molecular method detects DNA of parasite in samples and unable to distinguish viable from non-viable parasites (Holliman, 1994), the results obtained do not indicate the presence of living *T. gondii* in samples. However, it is well known that cyst forms of the parasite may survive several weeks between 1°C and 4°C and can be inactivated only in processes over 67°C or below -12°C (Aspinall *et al*., 2002). In our study, 64 meat-products samples (39%) including 16 salami, 13 sausages, 14 hamburgers and 21 kebabs were contaminated with *T. gondii*. Our results are consistent with several previous reports. A similar study that was conducted by Da silva in Brazil showed that *T. gondii* DNA was present in 27.14% of sausage samples (da Silva *et al*., 2005). A recent study in Turkey showed the presence of *T. gondii* was 19% in fermented sausage samples (Ergin *et al*., 2009). Another study by Aspinall *et al.* (2002) showed that 38% of meat sample were *T. gondii* positive by PCR detection and determined a much higher level of contamination that may be related to various prevalence of infection. These findings may be related to prevalence of infection in meat-producing animals or different eating habits. Our study revealed that amplification of 5' and 3' flanking region were successful respectively in 31.7% and 40% of samples. Similar findings have been observed in some other studies. In a Spanish study (Fuentes *et al*., 2001), no amplification of 5' and 3' regions was observed in 6% and 18%, whereas in a study were carried out by Gallego *et al.* (2006) in Colombia, amplification were failed in 9% and 57% for 5' and 3' flanking regions. It is thought that polymorphism in SAG2 flanking regions might be the cause of this phenomena. In summary, our results confirm the presence of *T. gondii* DNA in meat products. Therefore, the potential risk of the transmission of the disease through *T. gondii* containing meat should still be considered a public health threat. In view of recent developments in the epidemiology of the disease, it is suggested that not only pregnant women should be addressed but the whole population should be informed how to prevent infection.

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REFERENCES


