

## Investigation on *Toxoplasma gondii* in Surface and Drinking Water Samples from Amasya by Nested Polymerase Chain Reaction

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### Abstract

Occurrence of *Toxoplasma gondii* was investigated in surface and drinking water samples collected from Amasya by nested PCR. Total 120 surface water samples and 20 drinking water samples were flocculated by Aluminum Sulfate and they were purified sucrose-gradient. All of the water samples were used for DNA extraction and then, nested PCR were performed for them. While 48 out of 120 water samples (40%) were found positive for *T. gondii*, there were no any contamination in 20 drinking water samples for *Toxoplasma*. The water samples taken from Tersakan (Suluova), Tersakan (Karasu), Tersakan last and Yesilirmak (Tasova) stations were positive for *Toxoplasma*. This study is first report on investigation *T. gondii* in Amasya by using Nested PCR. Amasya Province has extensive agriculture and animal husbandry and also, it has insufficient waste water treatment plants. Since River Yesilirmak is used for agricultural terrain irrigation, this study is important in maintaining public health of this region

**Keywords:** *Toxoplasma gondii*, Nested PCR, Surface water, Black Sea

## INTRODUCTION

*T. gondii* have epidemiological spreaded to humans through oocyst-contaminated water [1]. Contaminated water could be a risk factor for human toxoplasmosis because all the *Toxoplasma* hosts need water to survive. A single one ingested with contaminated food and water may be enough to infect humans and animals [2].

PCR is more preferable than the conventional mouse bioassay for the detection of *T. gondii* oocysts in water, since it takes short time to detect such as from weeks to 1 to 2 days [3,4]. PCR-based molecular methods has becoming a favored technique because the molecular diagnosis is more sensitive and cost-effective than the conventional methods [5].

In the present study, we investigated the prevalence of *T. gondii* in the surface and drinking water samples from Amasya Province at Middle Black Sea area by Nested PCR.

## MATERIALS AND METHODS

### Study area

Havza, Suluova, Kanlıdere, Bogazkoy, Karasu, Stream Tersakan last and Amasya bridge, Safmaya, Durucasu, Tasova (Yesilirmak River) in the Province of Amasya have been selected as the ten water sampling sites (Fig.1)

Amasya is located in the middle of the Black Sea region. Amasya has the climate of a transition between the climate of the Black Sea and land's climate. Sixteen percent out of forest and 54% of pasture and 30% planting of the Province Amasya are available [6].

Yeşilirmak River Basin goes through the city center of Amasya and it combined with Tersakan River coming from Lake Ladik and Kelkit River on the Tasova-Erbaa Broughs. Then they are poured to the Black Sea in Carsamba Brough of the Province Samsun. Tersakan River have contaminated with waste of Havza and Suluova Broughs and then it mixed with Yesilirmak River.

**The collection of water samples**

One hundred-twenty water samples were collected every month regularly from Amasya in the period between December 2010 and November 2011. Water samples were collected and processed as previously described by [7]. Briefly, ten liters of water samples in sterile plastic bottles were collected from sampling site. Dark glass bottles were used for each sample to treatment in Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> flocculation. Then all samples were concentrated as described by [7,8].

**DNA extraction**

*Toxoplasma* tachyzoites (strains RH) DNA was extracted by the QIAamp DNA Mini Kit (Qiagen, Germany) according to the modified protocol of [7] with the addition of 10 freeze-thaw cycles in liquid nitrogen. DNA was eluted in 50 µL TE buffer in a clean tube and kept at -20°C until PCR were performed.

**Nested PCR**

The nested PCR targeting 18S-rRNA gene was performed according to [9] and as it has been later applied by [8]. In this study, their protocols were performed by modifying. Both PCRs were performed in standard mixtures of 25 µL containing 10 µM of each primer, 200 µM dNTP of each dNTP, 1x PCR buffer containing 1.5 mM MgCl<sub>2</sub> (Qiagen), 5 U HotstarTaq DNA polymerase (Qiagen) 5 µL Q-solution (Qiagen) and the templates were subjected to an amplification program with a Peqstar thermal cycler consisting of an initial step at 95°C for 15 min, 30 amplification cycles (94°C for 1.5 min, 45°C at primary PCR and 45°C at secondary PCR for 1.5 min, 72°C for 1.5) followed by one cycle of 10 min at 72 °C. This nested PCR amplifies a 341-bp amplification product for *Toxoplasma*. PCR products were analysed in 1.5% agarose gel stained with ethidium bromide solution (1 µg/mL) and visualized under UV light.

**Nested PCR in spiked water pellets**

Ten *Toxoplasma* oocysts from the stock solution were added in 10% aliquots of the selected sample pellets. After the DNA extraction, nested PCR assays have been applied

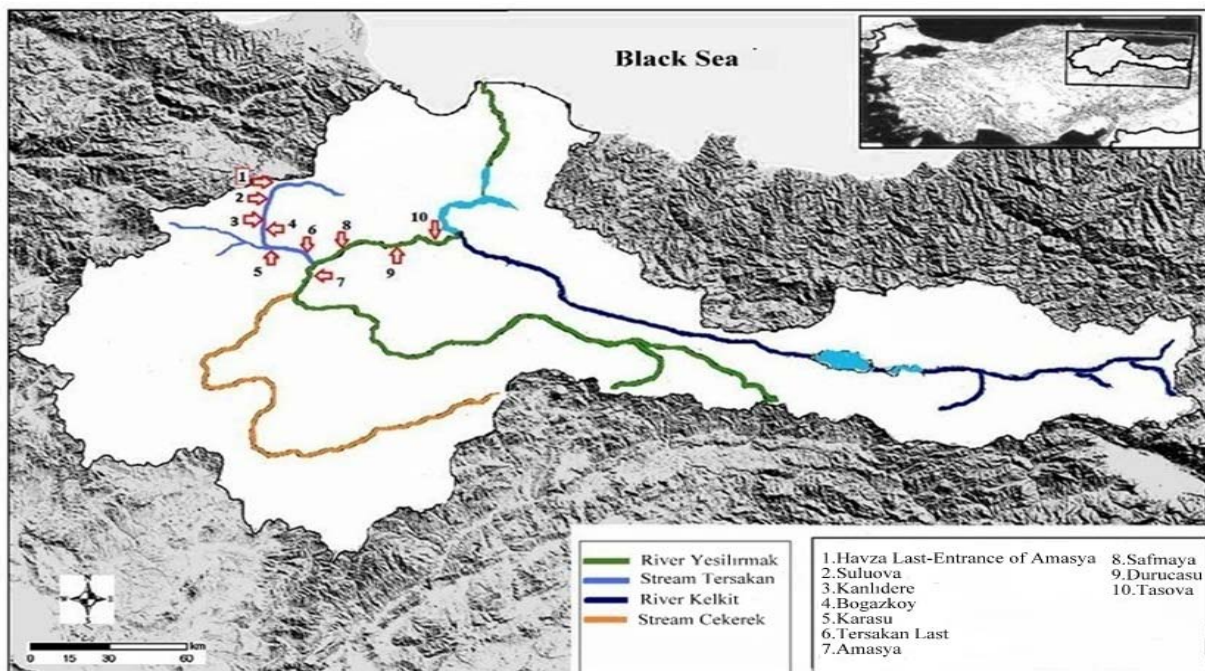
for all 20 spiked surface and 10 spiked drinking water samples sediments to determine efficiency of the nested PCR assays.

**RESULTS AND DISCUSSION**

*Toxoplasma* DNA were detected in 48 (40%) of 120 surface water samples from Amasya by nested PCR for a year. The water samples taken from Tersakan (Suluova), Tersakan (Karasu), Tersakan last and Tasova (Yesilirmak) stations were positive for *Toxoplasma*. The radomly selected pellets of 30 (20 surface and 10 drinking) spiked water samples from the Province of Amasya were tested by nested PCR for demonstrating positive controls. Fifteen of 30 spiked water samples were positive by nested PCR (Table1).

**Table 1.** The result of nested PCR for detection of *T. gondii* from spiked water samples collected from Amasya, Middle Black Sea area in Turkey

Investigated site	Nested PCR (spiked surface water samples)	Nested PCR (spiked drinking water samples)
Havza	0/2	5/10
Suluova	2/2	
Kanlidere	0/2	
Bogazkoy-Tersakan	0/2	
Karasu	2/2	
Tersakan end	2/2	
Amasya Bridge	2/2	
Safmaya	0/2	
Durucasu	0/2	
Tasova	2/2	
Total (%)	10/20 (50%)	5/10 (50%)



**Figure 1.** Location of study sites.

The waterborne toxoplasmosis outbreaks has cause to an increase in research interest in this area. The feces of felids are spreaded *T. gondii* oocysts into the environment and oocysts can survive in terrestrial and aquatic environments. Since *T. gondii* oocysts are highly resistant to chemicals and disinfections used in water industry, the risk of waterborne toxoplasmosis is remarkable. The chlorination, ozone treatment, and ultraviolet rays in water treatment plants are not block *T. gondii* oocysts [4]. Thereby, the consumption of drinking water contaminated by *T. gondii* oocysts has led to an increase human toxoplasmosis [10,11,12]. Since there is no rapid detection method for *T. gondii* oocysts in water or other environmental samples, detection of *T. gondii* oocysts in water is more difficult than that of other coccidian oocysts as described by [13].

According to [12] though *Cryptosporidium* oocysts and *Giardia* cysts detection are described in [14]. the detection of *Toxoplasma* oocysts are still problematic in water samples. However, there are several working groups that they described for the detection of *T. gondii* oocysts in water as [9,12,15].

PCR is increasingly important for detection of *T. gondii* compared with mouse bioassay. Since detection time of PCR is shorter than the other such as from weeks to 1–2 days as previously reported [4,9,16,17].

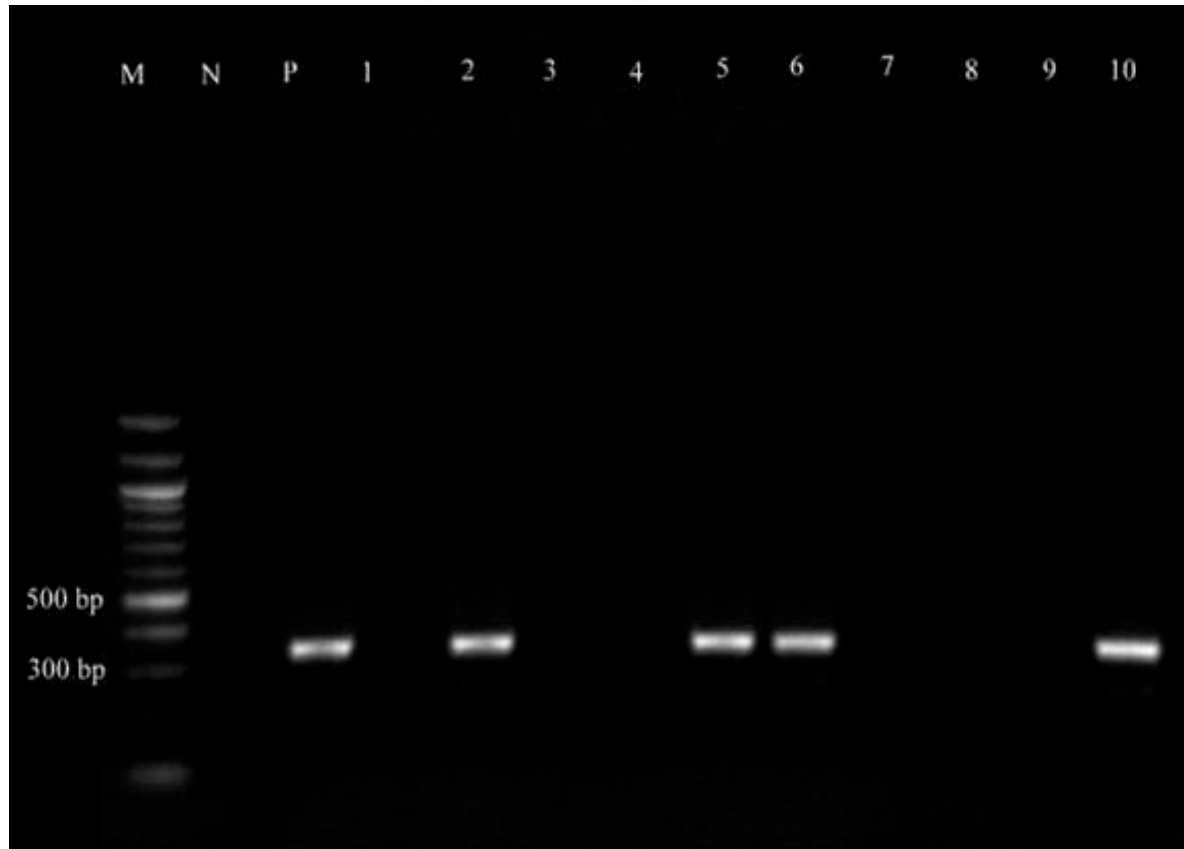
PCR assays affected by the negative from high concentrations of PCR inhibitors and low numbers of *T. gondii* oocysts in environmental samples [17]. The flocculation or sucrose floatation of water samples before DNA extraction are reduced PCR inhibitors and they can provide effective PCR detection of *T. gondii* oocysts in water [9,16,17].

In our study, a total 120 surface water samples and 20 drinking water samples were examined by nested PCR of the 18S-rRNA gene. Forty-eight out of 140 (34.7%) of the tested water samples were positive by nested PCR. Thirty (20 surface and 10 drinking water samples) spiked water samples from Amasya Province were tested by Nested PCR and Fifteen of 30 spiked water samples were found positive by nested PCR.

In the study by [15], a total number of 52 natural samples and 26 spiked water samples were investigated by nested PCR of the 18S-rRNA gene. 16 environmental water samples from Rostov area and 36 natural water samples from Sofia greater area were analyzed and seven out of 52 (13.5%) natural water samples were found positive by nested PCR. Ten spiked water samples from Sofia greater area and 16 spiked water samples from Rostov area were analyzed by nested PCR and 14 of 26 (53.8%) spiked water samples were found positive by nested PCR.

Thirty-seven of 482 (7.7%) environmental samples for detection of *T. gondii* oocysts by PCR analysis have been published by [18]. In addition, 114 drinking water samples were analyzed by PCR and the infection rate of *T. gondii* oocysts were 31 (27.2%) in water samples by [19].

In the present study, *Toxoplasma* DNA were detected in surface water samples from Amasya by nested PCR for a year. The contaminated four site Suluova, Karasu, Amasya end are in Stream Tersakan of Amasya and the other one is Tasova in River Yesilirmak of Amasya. The reason for contamination of Yesilirmak River and Tersakan Stream is mainly livestock. A very large part of the animal wastes which formed from the intensive livestock activities in especially Suluova Borough of Amasya are directly poured to Tersakan Stream and this also constitutes a very intense



**Figure 2.** Nested PCR detection of *Toxoplasma gondii*

M: 100 bp ladder; N: negative control (distilled water); P: positive control (*T. gondii* strains RH); lanes 1–10: all water samples; lane 2: Tersakan (Suluova); lane 5: Tersakan (Karasu); lane 6: Tersakan last; lane 10: Yesilirmak (Tasova)

pollution in decreasing flow rate of Tersakan Stream. Because almost all of the water from Tersakan Stream is used in irrigation water in summer, Tersakan Stream's flow rate is decreasing and it completely dries. At the same time the various types of vegetables/fruit wastes and other degraded solid waste are poured into the stream bed. Agricultural pollution mix an easier way to rivers because of inclined terrain. The barn manure in Yesilirmak Basin in a year is approximately 28,000 tons. River Yesilirmak are affected from intensive cattle, sheep and poultry farming activities in Amasya [20]. Yesilirmak River and Basin have continually been contaminated with waste water. Since the people in this area benefit from River Yesilirmak for utility water and in agriculture irrigation, water resources in here needs to be checked regularly to protect themselves from infection depending on the water pollution. In addition, waste water should not receive to basin or after the completion waste water treatment, they should be discharge in to basin to protect the health of the people living in this region.

## CONCLUSION

In conclusion, this present study demonstrated that occurrence of *T. gondii* DNA in surface and drinking water samples in the Amasya Province at Middle Black Sea area. There is little information about waterborne protozoan in this study area and in addition no any requirements for the routine detection of these parasite in surface water supplies in Turkey. For this reason, this study will help us to understand the prevalence of *Toxoplasma* in this investigated region and we also suggest that people living in this area should avoid to use surface water. If they have to use untreated water, at least they can boil that water to eliminate *T. gondii* oocysts.

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