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Original paper

Occurency of *Giardia duodenalis* assemblages in river water sources of Black Sea, Turkey

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ABSTRACT

A total of 420 environmental water samples and 120 drinking water samples from 45 different sampling sites of the Black Sea in Turkey were collected between 2012 and 2014. Genomic DNA was isolated from all the investigated water samples and comparatively analyzed by Loop-mediated isothermal amplification (LAMP) of the elongation factor 1 Alfa (EF1α) gene, and by nested Polymerase Chain Reaction (nPCR) of the small subunit (SSU) rRNA and semi-nested PCR (snPCR) of the glutamate dehydrogenase gene (GDH). 141 (58.7%), 125 (52.1%) and 120 (50%) samples respectivelly were positive by each method.

Out of 240 environmental samples collected from 25 sites of Samsun Province have been found positive for *G. duodenalis* by LAMP, nPCR and snPCR, respectively. 55 (30.5%), 50 (27.8%) and 47 (26.1%) of 180 environmental samples collected from 20 other sampling sites of Giresun Province were positive for *Giardia* by LAMP, nPCR and snPCR, respectively. Five PCR products from different samples of the Giresun Province and 10 other samples from the Samsun Province were found positive for *G. duodenalis* assemblage B. Five PCR products from Giresun Province and 5 samples from Samsun Province were found positive for *G. duodenalis* assemblage A.

This is the first report about *G. duodenalis* assemblages A and B from water samples investigations in Black Sea of Turkey.

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1. Introduction

Giardia is one of the common flagellate protozoan that can be transmitted by the fecal-oral route. Human gastroenteritis with this protozoan can be caused by contaminated water and food or by person-to-person contact (Adam, 2001; Barbosa et al., 2008; Baque et al., 2011). The genus *Giardia* has six known species such as *G. agilis*, *G. muris, G. microti*, *G. psittaci, G. ardeae* and *G. duodenalis*. Of these, *G. duodenalis* is a parasite of humans as well as the other mammals and it has cryptic species which includeseven genotypes from assemblages A to G. *G. duodenalis* assemblages A and B that have sub-assemblages reported as "A-I", "A-II", "B-III" and "B-IV" infect humans and animals (Karanis and Ey, 1998; Monis et al., 2003; Cacciò and Ryan, 2008; Plutzer et al., 2010). *G. duodenalis* assemblages A and B usually have been found in all investigated waste waters (Smith et al., 2006; Plutzer et al., 2010). Classical microscopic methods are not sufficient to indicate differences between species, genotypes and subtypes. For this reason, the molecular methods are required to demonstrate these differences. Although there are some studies on the prevalence of *Giardia* spp. from animals and humans in Turkey, there are no information on assemblages and/or genotypes of *Giardia* spp. in water supplies. The first genetic characterisation of *Giardia* spp. obtained from water samples in Black Sea of Turkey has been performed in the present study.

According to the recent published papers the assemblage A is identical with the species *G. duodenalis*, which is common in humans and other primates and a wide range of mammals and the assemblage B is identical with the species *G. enterica*, which is common in humans and other primates, dogs, cats, and some species of wild animals (Thompson and Monis, 2012; Thompson and Ash, 2016). Genetic substructuring and intraspecific variation has been reported in both zoonotic species referred above (Wielinga et al., 2015). Some *Giardia* species are host specific and others have low host specificity and capable of zoonotic transmission. The transmission is exacerbated via water and food. Wildlife has been found often infected with *Giardia* (Thompson and Ash, 2016). Many studies confirmed the distribution of *Giardia* spp. in water supplies and

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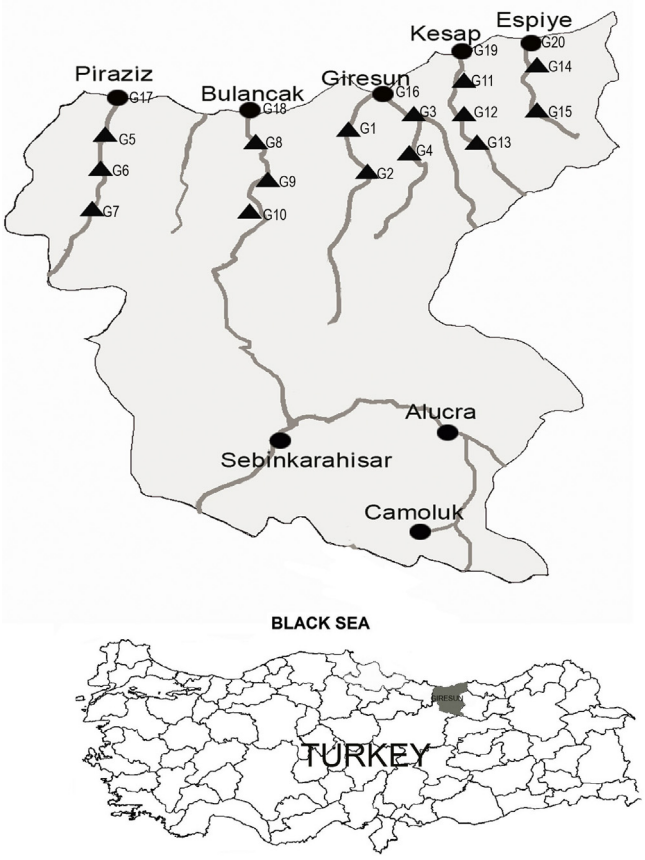

Fig. 1. The map of sampling sites from Giresun Province in The Black Sea.

their removal by the water treatment seems to remain a big challenge (Karanis et al., 1998, 2007; Baldursson and Karanis, 2011).

In the present study, we applied Polymerase Chain Reaction (PCR) and LAMP assays for the detection of *Giardia* spp. cysts in environmental water samples from river water sources in different provinces of the Black Sea region, Turkey.

2. Materials and methods

2.1. Geography and samples' collection

The collection of water samples from Black Sea sites (Figs. 1 and 2) was performed in the autumn of the years 2012 and 2013 and in the spring of the years 2013 and 2014. 20 sampling sites from Giresun Province have been selected for this study: a) River Aksu, River Bogacık, River Batlama, River Buyukgure were located near the center of Giresun; b) Three sampling sites such as the center of Piraziz Borough, Cayıragzı River and Keloglu River, which are close to Piraziz Borough; c) River Bulancak, River Karadere, River Incivez in Bulancak Borough; d) River Gelivera and River Yaglıdere in Espiye Borough and River Yolagzı, River Kesap, Entrance bridge of River Kesap in Kesap in Giresun Province (Fig. 1).

25 sampling sites have been selected from Samsun Province: a) River Mert, River Kurtun were located near the center of Samsun; b) Rivers Akcay, Milic and Terme, which are close to Terme Borough; c) Rivers Yesilırmak and Irmaksırtı in Carsamba Borough and Rivers Gelemen, Selyeri, Kirazlık in Tekkekoy; d) River Kızılırmak in Bafra Borough and 5 additional sampling sites for untreated drinking and usage water in Samsun Province (Fig. 2).

2.2. Sample's volume and giardia spp. cyst's purification

Ten liters samples of water have been taken from different locations of the Giresun and Samsun Provinces. They were collected in sterile plastic bottles without chemical additives and were immediately transferred to the lab for processing. The water samples were used for $Al_2(SO_4)_3$ flocculation in the first step of purification of cystsand in the second step cysts have been concentrated by sucrose flotation as described by Karanis and Kimura (2002) and applied in previous studies by, Karanis et al. (2006) and Koloren et al. (2011).

2.3. DNA extraction

DNA isolation was conducted from the purified samples by the manufacturer protocol of QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) as described previously by Plutzer et al. (2008), Koloren et al. (2011). Accordingly, after lysis buffer has been added to water pellets, freeze for 1 min and thaw in boiling water until dissoling were applied to this mixture for 15 times in a row in liquid nitrogen. DNA was eluated in 50 μl of Tris-EDTA buffer and saved at −20 °C until used in molecular methods such as nPCR and LAMP and snPCR. 100 μl total DNA with 50% of the sucrose floatation water concentrates was performed for DNA extraction.

2.4. PCR assays

2.4.1. nPCR based on the (SSU)rRNA gene

(SSU)rRNA gene for *Giardia* was amplified by nPCR as previously described by Appelbee et al. (2003) and Plutzer et al. (2008). A 292 bp fragment of the 18S rRNA locus was obtained. The PCR was performed in 25 μl final volumes that contained 100 pmol of forward and reverse primers, 25 mM dNTP, 10×PCR buffer containing 1.5 mM $MgCl_2$, 5×Q solution, and 25 mM $MgCl_2$, Hotstar Taq DNA polymerase 5U/μl. A Peqstar thermal cycler was used for amplification that consisted of 40 cycles (95 °C for 30 s, 63 °C at both PCRs for 45 s, 72 °C for 60 s), followed by 1 cycle of 10 min at 72 °C. PCR products were analyzed in a 1.5% agarose gel electrophoresis stained with ethidium bromide solution. All positive samples were subjected to sequence for genotyping.

2.4.2. snPCR for GDH gene

All water samples were subjected to amplify the GDH gene as described by Read et al. (2004). A 432-bp fragment of GDHgene was amplified. The PCR was performed in 25 μl standard mixtures that included 100 pmol of forward and reverse primers, 25 mM dNTP, 10×PCR buffer containing 1.5 mM $MgCl_2$, 5×Q solution, and 25 mM $MgCl_2$, HotstarTaq DNA polymerase 5U/μl. A Peqstar thermal cycler was used for amplification that consisted of 35 cycles (94 °C for 60 s, 55 °C at primary and secondary PCR for 45 s, 72 °C for 60 s), followed by 1 cycle of 10 min at 72 °C at the primary PCR. PCR products were visualized in a 1.5% agarose gel electrophoresis stained with ethidium bromide solution.

2.5. Giardia LAMP assay for EF1α gene

LAMP assay was performed for the detection of *Giardia* spp. as reported by Plutzer and Karanis (2009). The components for the LAMP assay in a final volume of 25 μl reaction mixture for *Giardia* were: reaction buffer [40 mM Tris-HCl, 20 mM KCl, 16 mM $MgSO_4$, 20 mM $(NH_4)_2SO_4$, 0.2% Tween 20, 1.6 M betaine and 2.8 mM each deoxynucleoside triphosphate], 8 U Bst DNApolymerase. 1.6 μM of each of FIP and BIP, 0.2 μMof each of F3 and B3, 0.8 μM of each of LPB and LPF, 2.8 mM of each dNTP, 1.6 M of betaine, 20 mM of Tris–HCl (pH 8.8), 10 mM of KCl, 10 mM of $(NH_4)_2SO_4$, 16 mM of MgSO4, 0.2% Tween 20. After the reaction mixture was heated at

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