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Prevalence of *Cryptosporidium* species in water supplies of Amasya, Middle Black Sea, by Acid-Fast staining methods

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Abstract

The goal of this study was to investigate the prevalence of *Cryptosporidium* spp. in environmental and drinking water supplies in Amasya area, Middle Black Sea, Turkey by Acid-Fast staining methods.

A total of 100 water samples, including drinking and river water were collected and investigated for the detection of *Cryptosporidium* oocysts. All water samples were collected on a monthly basis from up to 10 discrete sampling sites in Amasya and its surroundings and boroughs in the period between September and August 2011. All water samples were purified by $Al_2(SO_4)_3$-flocculation for water pellets. The resulted pellets were stained by Acid- Fast stain. Subsequently, all collected water samples were directly screened light microscopically for *Cryptosporidium* oocyst detection by Acid- Fast staining method. 78 (78%) out of 100 water samples were positive for *Cryptosporidium* spp, whereas there were not *Cryptosporidium* oocyst in drinking water by Acid- Fast stain.

In this study has point out to assume contamination of *Cryptosporidium* spp.in this region and it provides to keep relation with the involving waterworks in Turkey to cut through the protozoa contamination problems

Keywords: *Cryptosporidium*, Black sea, Epidemiology, Acid-Fast

INTRODUCTION

Cryptosporidium spp. and *Giardia duodenalis* are major causes of diarrheal disease in humans and animals worldwide, and major causes of protozoan waterborne diseases [1]. These parasites are transmitted through contaminated water and food, in addition to the classical fecal–oral route. Transmission is sustained by both zoonotic and anthroponotic cycles [2, 3].

Cryptosporidium spp. have a complex, monoxenous life-cycle where all stages of development occur within one host. Infection begins when oocysts are ingested. As little as one oocyst can produce infection in a susceptible host [4]. Mature oocystscontain 4 sporozoites and excystation (to liberate sporozoites) is possibly triggered by a combination of environmental conditions such as pH, bile salts, carbon dioxide and temperature [5]. The severity and longevity of *Cryptosporidium* infections are directly related to the immune status of the host. Clinical sign are generally more chronic and severe in the immunocompromised and are not always confined to the gastrointestinal tract. Extra intestinal infection of the respiratory tract pancreatic duct, gallbladder and biliary tree have all been documented in human immunodeficiency virus infected patients [6].

The aim of this study was to investigate the occurence of *Cryptosporidium* spp. in environmental and drinking water supplies in Amasya area, Middle Black Sea, Turkey by Acid-Fast stain.

MATERIALS AND METHODS

Water sampling sites

Amasya is a city in the Middle Black Sea coast of Turkey with population of 85 thousand. Amasya city has 7 county boroughs such as Suluova-Taşova which have been selected as water sampling sites. The Black Sea Basin is the largest river basin in Turkey and a significant part of rivers in Turkey flow into the Black Sea. The Yesilirmak is one of the largest river in Turkey and a significant part of its flow into the Black Sea (Figure 1).

Water sample collection and oocysts concentration

All samples were obtained in the period between December 2010 and August 2011. The investigations involved collection of water samples from selected sampling sites in rivers Tersakan and Yesilırmak (Figure 1). River water samples were of particular interest due to cloudy water and the presence of animals for feeding in the investigated catchment areas**.**

Concentration of water samples by Al2(SO4)3-flocculation

All collected water samples around Amasya at the Middle Black Sea area from different sources were purified by Al2(SO4)3-flocculation as described by [7] and as it has been later applied by [8] and [9]. Ten litters of water from the catchments' areas were collected in sterile plastic bottles

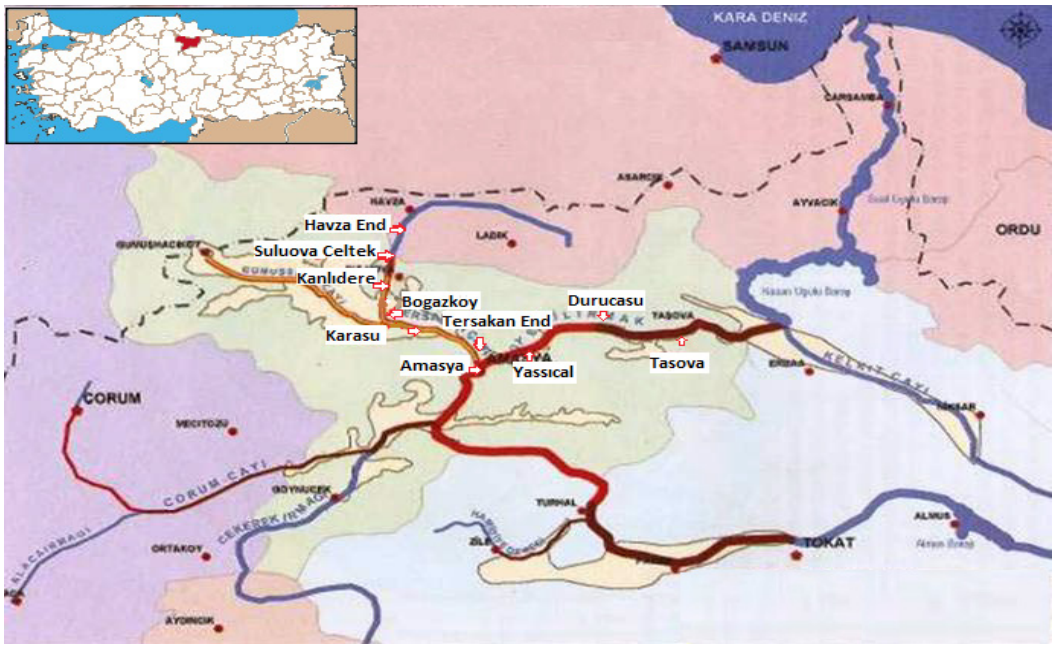

Figure 1. The map of sampling site

without chemical additives and were immediately transferred to the lab for processing. The collected water from each sample was decanted to dark glass bottle to perform flocculation. In summary, the water samples were overnight left to allow floc precipitation (pH5.4-5.8) after the addition of 10 mL Al2(SO4)3-solution. The next day the samples were concentrated and washed and consequently lysis buffer was added to disrupt the flocks.

Microscopic detection and identification of *Cryptosporidium* oocysts by Acid-Fast staining methods

For microscopic examination wet preparations in water pellet smear were prepared and examined for oocysts. Smears for modified acid fast staining were prepared by taking a pea sized material from the water samples by thin smear on clean glass slide. It was left to dry and fixed with 100% methanol. After fixation smear was flooded with cold carbol fuchsin and left for ten minutes. Smear was decolourized with 10% H_2SO_4 or TB decolorizer until colour ceased to flow. Smear was rinsed with water and counter stained with TB brillant green counter stain for 30 seconds. The preparation was rinsed again with plain water, dried and examined under oil immersion objective. Oocysts of Cryptosporidium appear as bright rose pink spherules against light blue background. The modified acid fast technique is a sensitive diagnostic method as recommended by [10].

RESULTS AND DISCUSSION

A total of 100 water samples from Amasya city and their boroughs were analyzed by Acid- Fast stain. Altogether 90 river water and 10 driking water samples were identified positive for the presence of Cryptosporidium cysts, when screened by Acid-Fast staining method under light microscopy. Table 1 presents the results from the occurrence of *Cryptosporidium* oocysts in the water samples collected during December 2010-August 2011 and analysed by Acid- Fast stain.

78 out of 100 samples were positive for *Cryptosporidium* (78%) when investigated by Acid- Fast stain. Interestingly, 78 river water samples, (representing a ratio of 86,66 %), out of 90 water samples examined, were found contaminated with *Cryptosporidium* oocysts by Acid- Fast stain. In contrast, no parasites were detected in 10 tap water samples from all samples. Summarizing the data on the occurrence of *Cryptosporidium* in water resources from Amasya city and its boroughs were reported in Table 2.

Cryptosporidium parvum oocysts stain as pale to bright pink spheres against a dark green or purple background. The oocysts are 4–6 µm in diameter. They are roughly the size of a red blood cell. To be clinically significant, oocysts should be readily identifiable on the slide and many high dry fields should have more than one oocyst at a time [11].

In our study, we observed oocysts under a light microscope, by observation of ten different fields with bright rose pink spherules against light blue background (Figure 2).

Cryptosporidium infects a wide range of vertebrate hosts including mammals, rodents, birds, reptiles and fish, and oocysts excreted by these hosts can be expected in our environment. Wildlife can also harbour their own host-adapted species, which may not be infectious to humans. While increasing the breadth of molecular studies is necessary to define the zoonotic potential of oocysts found in our environment, clearly, molecular methods are also necessary to determine the risk of human-infectious species and genotypes being present in water and in/on foodstuffs [12].

Historically, waterborne outbreaks of cryptosporidiosis, originating from the ingestion of contaminated potable waters have been better recognised than those originating from the ingestion of contaminated recreational waters. Of 325 water associated outbreaks of parasitic protozoan disease documented worldwide, [13] identified that Cryptosporidium was responsible for 50.8% (165) of these outbreaks, and that 23.7% (77) of reported outbreaks were caused by Cryptosporidium

Table 1. *Cryptosporidium* detection by Acid- Fast stain. assay in water samples collected from Amasya city center and Amasya province-Middle Black Sea

Sampling location (county borough)	Water type (total)	Investigation month	Number of positive/ examined samples by IFT (*)
Havza	River water(9) Top water(1)	December 2010-August 2011	7/9 0/1
Suluova-Çeltek	River water(9) Top water(1)	December 2010-August 2011	8/9 0/1
Kanlıdere	River water(9) Top water(1)	December 2010-August 2011	6/9 0/1
Boğazköy	River water(9) Top water(1)	December 2010-August 2011	9/9 0/1
Karasu	River water(9) Top water(1)	December 2010-August 2011	9/9 0/1
Tersakan and	River water(9) Top water(1)	December 2010-August 2011	9/9 0/1
Amasya	River water(9) Top water(1)	December 2010-August 2011	5/9 0/1
Yassıçal	River water(9) Top water(1)	December 2010-August 2011	8/9 0/1
Durucasu	River water(9) Top water(1)	December 2010-August 2011	8/9 0/1
Taşova	River water(9) Top water(1)	December 2010-August 2011	9/9 0/1
Total	River water(90) Top water(10)	December 2010-August 2011	78/100 0

Table 2. Summarized table on the occurrence of *Cryptosporidium* in water samples collected from Amasya city and county boroughs examined by Acid- Fast stain

Water type	No. of examined samples	Total no. of positive samples by Acid-Fast
Tap water	10	0
Subtotal % positive	10	0 (0)
River water	90	78
Subtotal % positive	90	78 (86,66)

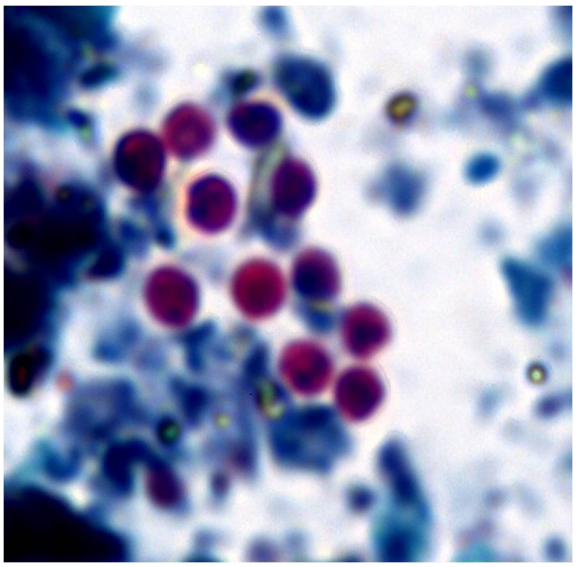

Figure 2. *Cryptosporidium* oocysts stained by Acid-Fast

sp. which either passed through filtered or unfiltered drinking water systems, or contaminated distribution systems in both small and large community water systems. Of the reported outbreaks of cryptosporidiosis, 50.3% (83) were associated with contaminated recreational water [13]. Swimming in contaminated waters and swimming pools is now recognised as an important transmission route for Cryptosporidium [12].

The number of parasites required to induce infection is relatively low. In fact, the infectious dose has been estimated to be as low as 10 *Cryptosporidium* spp. oocysts (Fayer R, Morgan U, Upton ,2000) *Cryptosporidium* is increasingly gaining attention as a human and an animal pathogen mainly due to its dominant involvement in worldwide waterborne outbreaks [1].

The discrimination of the *Cryptosporidium* species such as *C. parvum* type 1 (*C. hominis*) and *C. parvum* type 2 are required molecular techniques. Standard microscopy dedicates to us just supporting data regarding as prevalence *of Cryptosporidium* spp in investigated area, since İt is hard to discriminate oocysts from for all *Cryptosporidium* spp. by standard microscopy. For this reason, this study will help and basis for next further investigations about different species of *Cryptosporidium spp.* by molecular tools.

CONCLUSION

This present study provides the first report on detection of *Cryptosporidium* species from water supplies in Amasya at Mid -Black Sea by Acid Fast staining. Since there is no previous report about water-borne protozoan's in the investigated area, the present article will contribute not only to the initiation of protection measures for public health but also it will be the platform for further and more extensive studies in the Black Sea greater area. We suggest that strategies for minimization of risk such as focussed seasonal testing, greater adoption of treatment for the water, improvements with legislative powers for local authorities and financial incentives for water treatments.

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