

# The Protozoa and helminths in the water of Terme and Kocaman Boroughs of Samsun Province

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## Abstract

**Aim:** It was aimed to determine the presence of parasites and helminths in the environmental waters of the Terme and Kocaman districts of Samsun.

**Material and Methods:** In this study, 6 stations in Terme and 6 stations in Kocaman Miliç River areas were included.

The research was conducted between August 2012 - July 2013 and every month water samples were collected. The pellet part of the supernatant samples was examined on the same day by native-lugol methods. The samples were also examined by kinyoun acid fast, Weber's modified trichrome and trichrome methods. The samples were painted and evaluated according to parasitologic terms.

**Results:** As a result of water sample examination, 144 *Cryptosporidium* spp., oocysts 96 *Cyclospora* spp., oocysts 60 *Strongyloides* spp., larvae 36 *Microsporidia* spores, 48 *Blastocystis* spp. granular forms, 24 *Chilomastix* spp. trophozoits, 24 *Balantidium* spp. trophozoits, 60 hookworm eggs and 132 *Giardia* spp. cysts have been observed.

**Conclusion:** The animal husbandry made in those regions, direct discharge of the waste water pipes into the river, the usage of this water for irrigation and animal drinking and water cooling explain the presence of parasites in the water. As water is an agricultural, industrial and domestic requirement, it should be free of pathogenic microorganisms in accordance with the criteria related to its potential use. Water, which is a domestic requirement for agriculture and industry, needs to be purified from pathogenic microorganisms in accordance with the criteria for its potential use.

**Keywords:** Terme; Kocaman; Environmental Waters; Parasites.

## INTRODUCTION

Because, increasing population, developing industrialization and intensified water use are accelerating water pollution (1,2).

Approximately 200 million people are infected with waterborne diseases every year, and 2 million people die from these infections (3).

The bacteria and viruses and other pathogenic organisms that cause contamination of water hygiene, often have been transported with feces of diseased or carrier animals and humans (4,3). In addition, the drinking of water which is contaminated with stool or soil was reported to be the source for water-borne diseases, as well (5). The recommendations about purification and disinfection of water, ensure a healthy waste hygiene, health and hygiene education in order to prevent diseases that can be transmitted through water (3). Considering factors such as the social, structure agriculture, and livestock culture and the inadequacy of environmental conditions in the

Black Sea Region which receives abundant rainfall due to its climate characteristics, parasitic diseases in this area threaten public health (6).

In literature, there is no information about the epidemiology of the parasites in the waters of the Terme and Kocaman districts. During the transport of rainwater to streams, lakes and rivers in the Black Sea Region, which receives abundant rainfall, rapid spread of water pollution may be possible. In this study, we aimed at, determination of protozoa and helminths in water samples taken from Terme stream and huge Milic River in the province of Terme, Samsun.

## MATERIAL and METHODS

The specific regions on Milic River and Terme Tea located in Kocaman town of Terme have created the universe of work.

The location of both distincts near the center and sewage

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spill regions and residential areas where intensive animal husbandry were taken into account for water samples selection. Before the start of work places to take water samples were determined by going to the specified areas Six stations were identified, across the sewer pipe (T-1), under the bridge close to Pazar Mosque (T-2), Saray village outlet behind the fountain (T-3), Dibekli village grocery store (T-4), under the bridge near Mescitli village (T-5), Tuesday market (T-6). Six sampling sites of Milic River of Kocaman District were, the village entrance at the beginning of the river (K-1), separation of the two arms of river (K-2), under the central bridge (K-3), Kocaman Borough outlet (K-4), the central under bridge of Huseyin Masjid (K-5), the second bridge near Huseyin Masjid (K-6) Figure 1.



**Figure 1.** Map of Samsun province Terme and Hüge rivers (prepared by modifying with Gülabi (7)).

This research was carried out between August, 2012 and July 2013. A total of 144 water samples were collected on certain dates of each month. The water samples were collected when the weather was dry and before afternoon.

The clarity, stillness and fluidity of water were noted during the sampling dates. Water samples were taken from one meter away and at least about 30 cm depth of the stream. The examination and collection of water samples were conducted by the information resources (8,9). 5 liters of water from 12 different stations on Terme Tea and Kocaman River was collected with clean plastic containers every month.

For the precipitation of the materials in the water, the samples were kept in a dark condition for about 1 hour. Paying attention to the mixing of the resulting precipitate with the upper liquid part the liquid part was thrown with the help of the U-pipe. The resulting precipitate of approximately 200 ml was placed in 50 ml centrifuge tube in uniform and equal state and they were centrifuged 1000 rpm for 10 min at 4 °C. The supernatant was discarded and remaining pellet was collected into one tube. Then prepared samples were examined by native- lugol method on the same day. In addition, the smear water samples were prepared and they were checked by Kinyon's acid fast and Weber's trichrome and modified trichrome methods. The positive controls were also stained in each stain and evaluations were made accordingly.

**RESULTS**

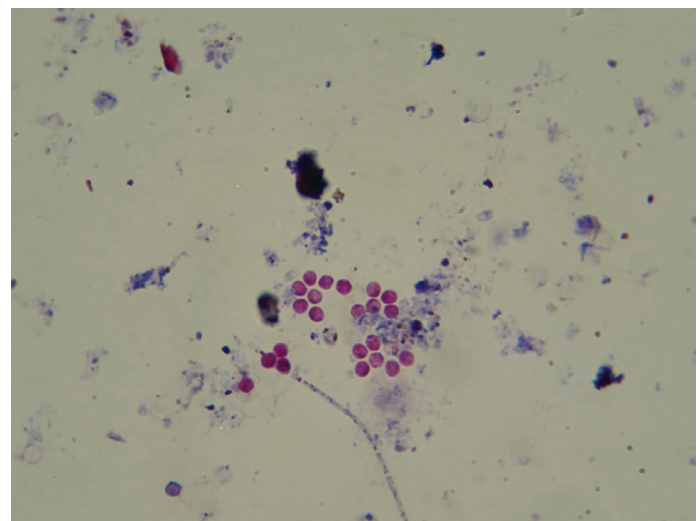
In this study research, 144 *Cryptosporidium* spp. (Figure 2) 96 *Cyclospora* spp. (Figure 3), 60 *Strongyloides* spp. (Figure 4), 36 *Microsporidia* (Figure 5), 48

*Blastocystis* spp, 24 *Chilomastix* spp., 24 *Balantidium* spp, 60 Hookworm (Figure 6) and 132 *Giardia* spp. Have been found positive in the examined water samples.

The distribution of parasites in the interference region is given in table 1.

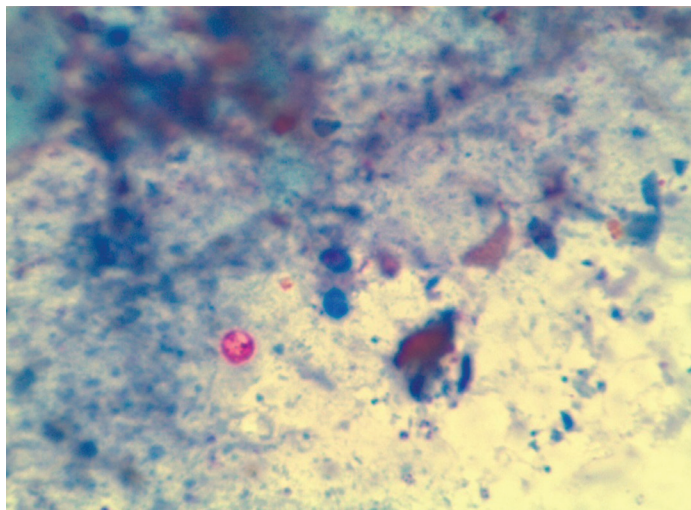
The parasite positive water samples were kept for PCR in this study. Positive *Giardia* spp, and *Cryptosporidium* spp, samples were confirmed by PCR. However, PCR was not performed for other parasites. Table 1, Figure 2-6.

Parasites	STATIONS											
	TERME						KOCAMAN					
	-1	-2	-3	-4	-5	-6	-1	-2	-3	-4	-5	-6
<i>Cryptosporidium</i> spp.	2	3	1	0	4	2	1	2	4	0	6	
<i>Giardia</i> spp.		2	0	4	6	2	0	3	1	7		
<i>Cyclospora</i> spp.	0	2	2	3	2	0					0	
<i>Microsporidia</i>	-	-	-	-	-	2	-	0	-	-	4	
<i>Blastocystis</i> spp.	-	2	-	0	4	2	-	-	-	-	-	
<i>Strongyloides</i> spp.	-	2	2	-	-	-	1	6	9	-	-	
<i>Chilomastix</i>	-	-	-	-	-	-	4	-	-	-	0	
<i>Balantidium</i>	-	-	-	-	-	-	-	-	2	1	4	

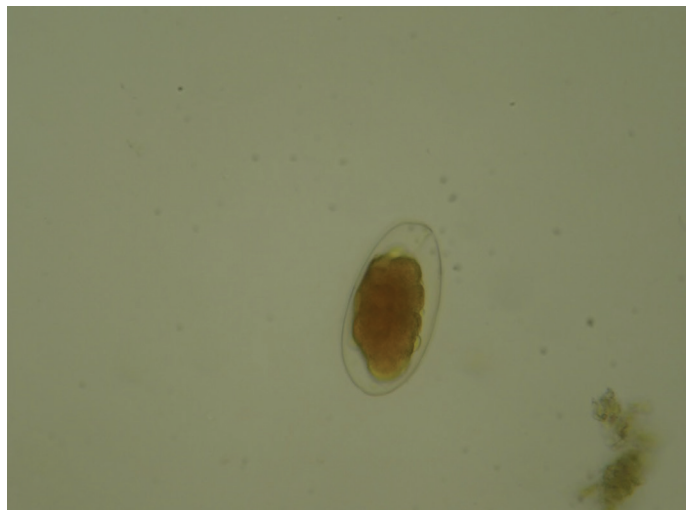


**Figure 2.** Kinyonun Acid Fast stain with *Cryptosporidium* spp. oocysts (100X)

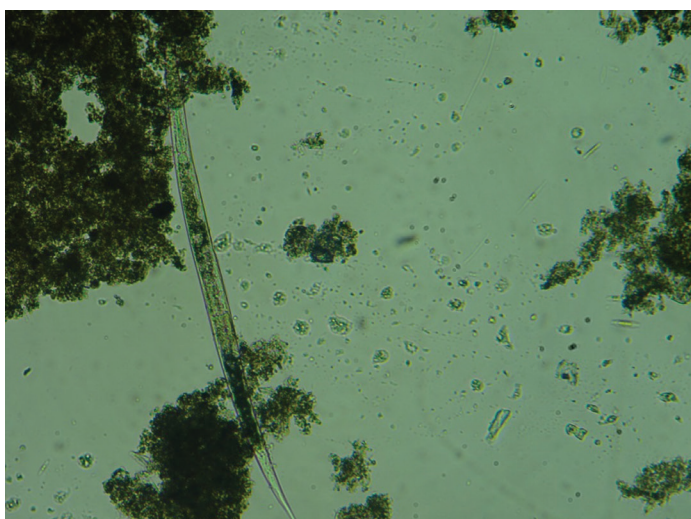




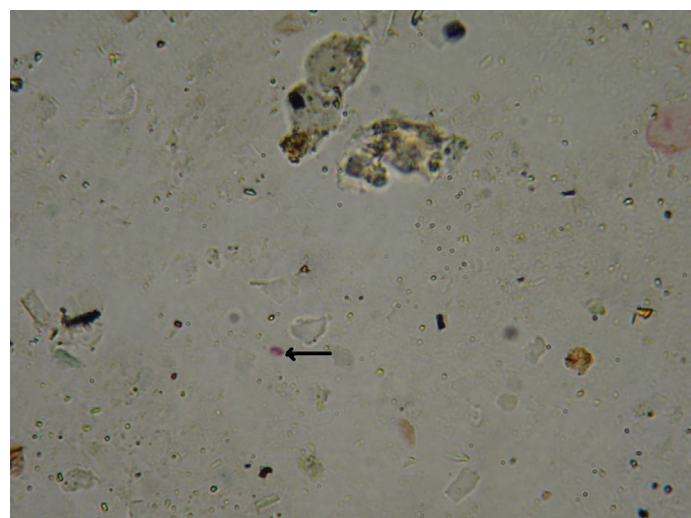
**Figure 3.** Kinyonun Acid Fast stain with *Cyclospora* spp. oocysts (40X)



**Figure 6.** Direct view with hookworm egg (40X)



**Figure 4.** Direct view with *Strongyloides* spp. larvae (10X)



**Figure 5.** Modified trichrome with stain microsporidia (100X)

## DISCUSSION

As a result of sample examinations, As a result of examining the samples *Cryptosporidium* spp. *Cyclospora* spp. *Strongyloides* spp., *Microsporidia*, *Blastocystis* spp., *Chilomastix* spp., *Balantidium* spp., *Hookworm* and *Giardia* spp. were found in this study. The oocysts of *Cryptosporidium* spp were identified to be 4.4 % in a survey in children with diarrhea (10). In another study, this parasite was found at the rate of 0,2 % in 455 people aged 15 (11). The prevalence of *Cryptosporidium* of % 1,54 (12) in Elazığ, % 11,8 in Sivas (13) and % 0,42 in İzmir (14) has been reported.

In a study conducted in Mersin, four children were infected with *Cryptosporidium* from drinking water at their school, while no infection in children of schools with clean water could be determined (15). In a study conducted in Mersin, infection occurred in four children in a school where *Cryptosporidium* was detected in drinking waters, whereas no infections were detected in children whose schools had clean water (11).

In Mersin Province, *Cryptosporidium* spp. oocysts were observed in drinking water, well water, waste water and sea water samples. The presence of *Cryptosporidium* spp. Oocysts in drinking water, potable water, waste water and sea water was researched in Mersin. *Cryptosporidium* spp. oocysts were found positive in five of the 44 drinking water samples (11.36 %), 1 of the well water, four of 19 waste water (21 %) and one of sea water samples respectively (2.85 %) (16). *Cryptosporidium* oocyst was detected in 5 of 44 drinking water samples (11.36 %), in 1 of the wells, 4 of 19 wastewater (21 %) and in 1 of the sea water samples (2.85 %) (12).

According to the result of a study conducted in Ordu province by Kolören and Kaya (17), The prevalence of *Giardia lamblia* was 61.3 % in Mesudiye, 52 % in Unye, 40.7 % in Korgan, 31.8 % in Fatsa, 30 % in Ulubey; 29.4 % in

Perşembe while the prevalence of *Cryptosporidium* spp. was 63.15 %, 54.3 %, 37.5 %, 36.8 %, 33.3 %; 31 % in Unye, Fatsa, Mesudiye Perşembe, Ulubey, Korgan, respectively.

In addition, *Cryptosporidium* spp. oocyst was determined in 1.13 % of a total of 440 drinking water resources in Van Province as described by Cicek and et al. (18).

The most common Protozoal infection to humans is *Giardia*. In temperate regions, 2-5% in industrial countries, and 20-30% in developing countries. (4). The incidence of *Giardia* in research conducted in different regions of our country, ranged from 1.9 to 37.7% (19). In our country, the incidence of the agent varies between 1.9% and 37.7% *Giardiasis* has been reported at 41% rate among food-borne parasitic outbreaks between 1989-1992 years by the Center for Disease Control It has been reported by the disease control center that 41% of foodborne parasitic outbreaks occurred between 1989-1992 (20).

Mons et al. (21) evaluated the contamination of protozoons in river water used as a source of drinking in and around Paris. In the waterworks, Mons et al. (17) evaluated the contamination of protozoons of river water used as drinking water source in and around Paris. *Cryptosporidium* was found in autumn while *Giardia* was detected in summer. In the case of seasonal evaluations, for the positive examples they found *Cryptosporidium* in fall and *Giardia* in summer.

It has been identified that the other fecal bacteria are not associated with examined protozoan. Similarly, Wilkes et al. (22) investigated the relationship between *Cryptosporidium* oocysts, *Giardia* cysts, pathogenic bacteria, indicator bacteria and seasons in surface water of agricultural lands. Similarly, Wilkes et al. (18) examined the relationship between *Cryptosporidium* oocysts, *Giardia* cysts, pathogenic bacteria, indicator bacteria and seasons in the surface waters of the agricultural regions.

They observed high positive yield among indicator bacteria and parasites in autumn and winter period. *Cryptosporidium* was found positive 100% of all water sample collected 144 and *Giardia* spp. was at rate of 91.6%.

*Cyclospora* is found all over the world, especially in countries with tropical or subtropical climate Although *cyclospora* is seen all over the world, it is more common, especially in countries with tropical or subtropical climates. Transmission of this parasite is through direct fecal-oral or waterways (23, 24). *Cyclospora* outbreak has been reported from drinking water among the British troops in 1994 and in a small military troops from Pokhara/ Nepal (25). In 1994 a *cyclospora* outbreak from drinking water was reported among British soldiers and in a small military troop in Pokhara / Nepal and the parasite was isolated from drinking water (21).

The first case in our country was determined by Ram et al. (26), when they were doing a research on the etiology

of chronic diarrhea in AIDS patients. The first case in our country was detected by Koc et al. (22) in a case of chronic diarrhea etiology of a patient with AIDS. The study identified *Cyclospora* at a rate of 66.6% in 144 water samples, as well.

*Blastocystis* spp. is the most common protozoan found in stool samples of healthy and ill individuals (27). *Cryptosporidium* spp., *Cyclospora* spp., *Strongyloides* species, *Microsporidia*, *Blastocystis* spp., Hookworm and *Giardia* spp. have been reported in water of Giresun province (28). Also, of the 228 water samples examined in the surrounding waters of Samsun province, 180 are stream, 48 are drinking water and 142 *Giardia* sp., 132 *Cryptosporidium* spp., 56 *Cyclospora* spp., 38 *Microsporidia*, 47 *Blastocystis* spp, 38 *Entamoeba coli* cysti, 18 *Dientamoeba* spp., 9 *Chilomastix* spp., 9 *Strongyloides* spp., 6 hookworms have been reported (29).

The presence of *B. hominis* was detected at the rate of 3.4% in stool samples from patients admitted to hospital of Faculty of Medicine in Dokuz Eylul University, while this parasite was found at the rate of 2.8% between 2003-2004 years (30, 31). *Blastocystis* spp. were found 33.3% of surface waters in this study. It has been reported that this parasite might be infect to people by the way of water (32).

In the accessed sources, there are only two studies on *microsporidia* in the water in Turkey (28, 28) When examining study performed with stool samples, *microsporidia* positivity was detected in 226 (8.5%) of 2665 patients as described before by Karaman et al. (32), and 225 diarrhea patients (9.8%) by Turk et al. (33). The presence of *microsporidia* was detected in 781 adults at at a rate of 6.5% by Atambay et al. (34) and they found a significant relationship between determinations of parasites with indigestion. *Microsporidia* spores were detected at a rate of 7.8% in children as described previously by Calik et al. (35). *Microsporidia* spores was found at the rate of 25% in water samples.

## CONCLUSION

In conclusion, water which is needed in agriculture, industries and domestic use should be free from pathogenic microorganisms in line with the criteria for its potential use. According to the information obtained from this study, the determination of epidemiology of parasites in humans and animals in this region and public health studies proposals were presented.

*Conflict Of Interest Statement: There is no conflict of interest between researchers.*

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