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# Monitoring of *Cryptosporidium* Species in Water Supplies of Sinop, Black Sea, Turkey by Acid-Fast Staining Method

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**Abstract**

The prevalence of *Cryptosporidium* spp. in sea (48 samples) and drinking water (10 samples) from the Sinop province, Black Sea, Turkey were investigated by acid-fast staining method. Ten litre samples of water were collected over 12 months from December to November 2011 for *Cryptosporidium* oocysts detection by acid-fast staining method. *Cryptosporidium* oocyst were detected in 46 out of the 58 samples (79.31%; 2-46 oocysts per 1 litre) in the water samples from Sinop by acid- fast stain. When a portion of the sea water samples taken from different sources in Sinop city center were positive by acid-fast stain there was no any contamination in drinking water samples. This study point out the need for regular and better monitoring of water-borne protozoans in sea water in Sinop city. In addition, then we need to focus on the important point that are monitoring of water supplies by molecular analysis in order to specify whether the parasites asset in this samples are human pathogenic or not.

**Keywords:** Cryptosporidium, Public health, Black Sea, Epidemiology

## INTRODUCTION

It is well known that protozoan parasites of the genera Cryptosporidium (phylum Apicomplexa) and Giardia (subphylum Sarcomastigophora) are important causes of disease and morbidity in humans and of losses in livestock production. Clinically, these protozoans infect distal and proximal regions of the small intestine, occupying epicellular and extracellular intestinal niches, respectively, which affect hosteparasite interactions, pathophysiology and disease mechanisms [1,2].

*Cryptosporidium* is increasingly gaining attention as a human and an animal pathogen mainly due to its dominant involvement in worldwide waterborne outbreaks [3,4].

The aim of the present study was to evaluate the prevalence of *Cryptosporidium* spp. in the sea and drinking water samples of Sinop provinces at Black Sea area by acid- fast stain.

## MATERIALS AND METHODS

**Sampling area and oocysts purification**

All samples were collected the sea water samples from selected 4 sampling sites in Sinop from December 2010 to September 2011. All the points were located center of cities. All points were only sea water from Sinop. Ten litters of water from the sampling areas were collected in sterile plastic bottles with no chemical and they were moved to the lab for testing once in every one month. A total of 58 samples were tested for contamination of *Cryptosporidium* oocysts. $Al_2(SO_4)_3$-flocculation and sucrose gradient concentration as it has been applied by [4,5,6,7].were used to obtain water pellets.

**Microscopic detection of *Cryptosporidium* oocysts by acid-fast staining method**

The water pellets were used for wet preparations for *Cryptosporidium* oocysts microscopic detection. A pea sized material from the water samples were spread by thin smear on sterile glass slide for modified acid fast staining. It was left to dry and fixed with 100% methanol. The cold carbol fuchsin was left for ten minutes in to fixation smear.Smear was decolourized with 10% H2SO4 or TB decolorizer. After rinsed with water, smear was stained with TB brillant green counter stain for 30 seconds.The preparation was rinsed again with 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 standard method as recommended by [5, 8].

**Statistical analysis**

Descriptive data analyses were done using microsoft excel and hypothesis testing using SPSSv. 18. Simple unvariate logistic regression was used to screen each indicator organism. The odds ratio, 95% confidence interval, and p value for that indicator organism are reported where the associated p value was statistically significant or nearly statistically significant (i.e. near a value of 0.05). In addition, a multiple logistic regression model that included all variables was calculated [9].

## RESULTS AND DISCUSSION

A total of 58 water samples (sea and tap) from Sinop were analyzed by acid- fast stain. 46 sea water samples were identified positive for the presence of *Cryptosporidium* oocysts, when analyzed by Acid- Fast stain in Sinop (Fig.1).

The maximum of positive sea water samples were detected in point 1 from Sinop center with 32 oocysts per 1L in January. The point 2 of Sinop center were found positive containing 11 *Cryptosporidium* oocysts per 1 L in January and March. The occurrence of *Cryptosporidium* oocysts in sea water samples collected from point 3 of Sinop center was positive with 42 oocysts per 1 L in January. In the point 4 of Sinop city were found highly positive containing 46 positive per 1L at 12 months. It was observed that there was a significantly higher ($p<0.05$) incidence of positive *Cryptosporidium* spp. oocyst samples in January than the other monhts except of the December, February and March for Sinop (Table 1). In contrast, no parasites were detected in 10 tap water samples from Sinop.

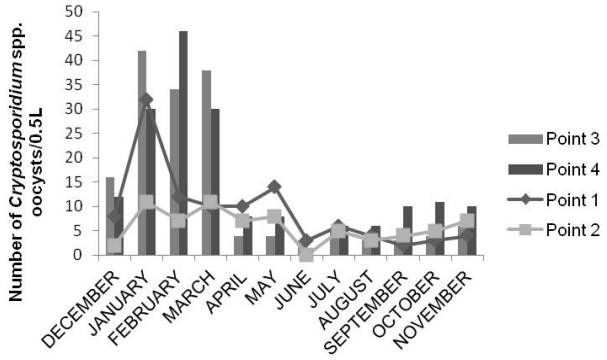


**Figure 1.** Prevalence of Cryptosporidium oocysts in the sea water samples of Sinop on a monthly basis

***Table 1.*** Multiple comparisons of *Cryptosporidium* spp. oocysts detection in sea water samples of Sinop on a monthly basis

| *Measured Cryptosporidium spp. oocysts* | | |
|---|---|---|
| *Investigation month* | | *(95% Confidence interval) p value** |
| *JANUARY* | *DECEMBER* | *19.25 (0.86-39.36); p=0.071* |
| | *FEBRUARY* | *4.0 (16.11-24.11); p=1.000* |
| | *MARCH* | *6,50(13.61-26.61); p=0.991* |
| | *APRIL* | *21.50* (1.38-41.61); p=0.028* |
| | *MAY* | *20.25*(0.13-40.36); p=0.047* |
| | *JUNE* | *27.25*(7.13-47.36); p=0.002* |
| | *JULY* | *23.25*(3,13-43.36); p=0.012* |
| | *AUGUST* | *24.75*(4.63-44.86); p=0.006* |
| | *SEPTEMBER* | *24.0* (3.88-44.11); p=0.009* |
| | *OCTOBER* | *23.0* (2.88-43.11); p=0.014* |
| | *NOVEMBER* | *22.0* (1.88-42.11); p=0.022* |

All water samples data showed a strong seasonal impact, with samples between December and November 2011. The seasonal variation in contamination of Sinop's water samples were that roughly over two times the level of contamination in winter compared to the other seasons. All in all positive for the presence of *Cryptosporidium* spp in sea water samples in winter, Point 3 had significantly higher than the other points in Sinop (Fig. 2 and Table 2). The average mothly rainfall and temperature in Sinop were illustrated in Fig. 3. As compared with Fig. 1 and Fig. 3, rainfall was significant factors affecting the level of contamination with *Cryptosporidium* in sea water in Sinop. The number of *Cryptosporidium* oocysts in winter and in the following spring were significantly higher than in summer and autumn. On the other side, lower temperature in winter and in spring enhanced the levels of contamination in Sinop.

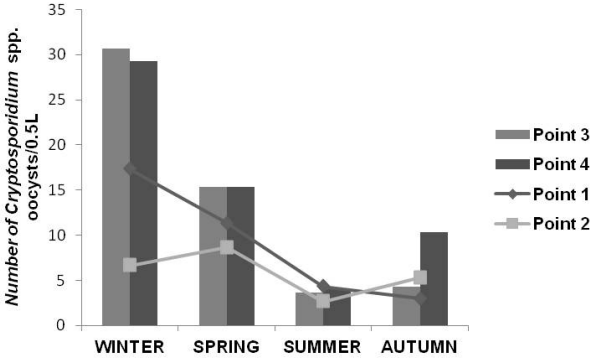


**Figure 2.** Prevalence of *Cryptosporidium* oocysts in the sea water samples of Sinop according to season

***Table 2.*** Multiple comparisons of *Cryptosporidium* spp. oocysts detection in sea water samples of Sinop according to season

| *Number of Cryptosporidium spp. oocysts/0.5L in the sea water samples of Sinop* | | | |
|---|---|---|---|
| *Season* | | *Cryptosporium spp.* | |
| | | *(max-min)* | *p value ** |
| *WINTER* | *SPRING* | *8.33 (4.46-21.13)* | *p=0.266* |
| | *SUMMER* | *17.33*(4.53-30.13)* | *p=0.008* |
| | *AUTUMN* | *15.24*(2.44-28.04)* | *p=0.019* |

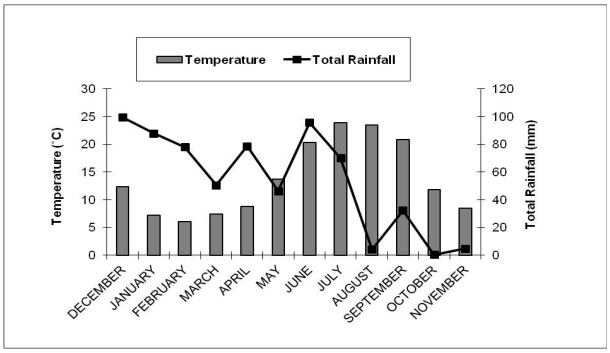


**Figure 3**. The average rainfall and temperature on monthly in Sinop

To identify the contamination in this areas can provide a proper basis for protective measures for public health. But, this study lacks the molecular analysis and we are not sure whether the parasites asset in this samples are human pathogenic or not. The (oo)cysts could be from cattle or dog source, but theirs parasites not human pathogenic species.

Water quality is a major concern all around the world, as water uses are threatened by generalized contamination resulting from human activities. This contamination concerns sediments as well as chemicals and microbiological components coming from industrial, municipal or agricultural point and non-point sources of pollution [10].

As described by [5] the origin of contamination parasites are in agricultural practices and possible in the dysfunction of sewage treatment plants during periods of heavy rainfalls. The Black Sea region has heavy rainfall, and this may cause faecally contaminated land with a pathogens to wash into surface and sea water. The safe drinking water use is aim of Turkey. However, there is a need for more information on waterborn protozoans and theirs diseases in here.

*Cryptosporidium* species infect humans and a wide variety of vertebrate animals. Because the oocyst stage responsible for transmission is ubiquitous in the environment cryptosporidiosis can be acquired through several routes: person-to-person contact, contact with companion and farm animals, and ingestion of contaminated food, drinking water and recreational water. Because this stage lacks distinctive morphologic features to clearly differentiate *Cryptosporidium* species, identification by microscopy is problematic for determining the species infecting humans or animals, the burden of disease attributable to different species, and the role of individual species in disease or transmission [11,12].

Classical methods are not adequate for the identification of some protozoon; also, molecular methods have to be used in designating the distinctions between the species. In recent years, one of the molecular methods which has been used frequently in the identification of protozoon is the technique of LAMP [13].

Molecular characterization is essential in identifying the parasite in infections, as well as aiding in the elucidation of possible sources of contamination and routes of transmission [14].

This study has documented the presence of *Cryptosporidium* species in sea and drinking water samples from Sinop province of Turkey by Acid-Fast stain. The present article will help to the initiation of protection measures for public health and this study demonstrate the need for better monitoring of water-borne protozoans in the recreational river and sea water in Sinop cities.

In here, we demonstrated that how common *Cryptosporidium* spp. are in this area by acid-fast stain as a classical method. But we need to use more sophisticated and sensitive molecular methods to detect genotyping of *Cryptosporidium* spp. Moreover, the determination of the species and genotype of these parasites by molecular assay contributes in the identification of host sources of *Cryptosporidium* spp.

## CONCLUSION

The present study point out levels of contamination by *Cryptosporidium* in sea and drinking waters from Sinop city by Acid-Fast stain. But there is a need to developing more sophisticated and sensitive molecular methods to detect water borne protozoans in this area for the identification. The present article will contribute to know overall pollution level by *Cryptosporidium* and it will be basis for future molecular assays.

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