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## Letter to the Editor

# Occurrence and Environmental Factors Associated with *Cryptosporidium* in South-Eastern Iran

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## Dear Editor-in-Chief

*Cryptosporidium* is a protozoan parasite causing significant waterborne illnesses, posing a primary concern for water treatment and public health (1). The seroprevalence in these regions is 25–35%, especially in children (2). As of 2021, 44 species of *Cryptosporidium* have been validated, 20 of which can infect humans (3). *C. hominis* and *C. parvum* are responsible for most human infections. Environmental factors such as pH, water temperature, turbidity, dissolved oxygen, salinity, and organic matter influence *Cryptosporidium* survival and presence (4).

This study was conducted from April 2021 to August 2022, via collecting water samples from Zahedan and Zabol cities in southeastern Iran's Sistan and Baluchistan provinces. Random sampling was performed at 80 locations in Zabol City, including surface, tap, bottled, well, and wastewater sources (5).

This study was approved by the University Research and Ethics Committee (code: http://ethics.research.ac.ir/IR.ZBMU.REC.1398.159).

Health and safety protocols were implemented (6). A minimum of 10 liters of water was collected for environmental samples, following the EPA Method 1623.1 (7). The samples were filtered through a membrane with a 2-3 µm pore size using the Centrifugal Vacuum Filtration method. Pellets trapped in the filter were removed and centrifuged to concentrate the oocysts (8).

The modified Ziehl-Neelsen method using microscopy identified the oocysts by staining them with carbon fuchsin, destaining them

with alcohol, and counterstaining them with methylene blue (9).

Of 180 water samples, 35 were positive for *Cryptosporidium* oocysts, 142 were negative, and three were suspicious. *Cryptosporidium* was most frequent at pH 7. Table 1 shows a significant difference between water temperature and the presence of *Cryptosporidium* oocysts, with more oocysts observed at temperatures between 20 and 30°C ($P$<0.001, TUKEY test) oocysts, with more oocysts observed at temperatures between 20 and 30°C ($P$<0.001, TUKEY test).

**Table 1:** Frequency and comparison of positive(A), suspected(B), and negative(C) *Cryptosporidium* parasite cases according to water PH, with a significant relationship between positive and negative samples

| *PH* | *Result* | *No.* | *Mean* | *Std. Deviation* | *95% Confidence Interval for Mean* | | *p-value* | *Post-Hoc* |
|---|---|---|---|---|---|---|---|---|
| | | | | | **Lower Bound** | **Upper Bound** | | |
| A | Positive | 35 | 7.3257 | .19303 | 7.2594 | 7.3920 | $P$<0.001 | A>C |
| B | suspicious | 3 | 7.3667 | .15275 | 6.9872 | 7.7461 | | |
| C | Negative | 142 | 7.0963 | .15489 | 7.0706 | 7.1220 | | |
| | Total | 180 | 7.1454 | .18795 | 7.1178 | 7.1731 | | |

*. The mean difference is significant at the 0.05 level.

Regarding the relationship between *Cryptosporidium* spp., pH, temperature, and physical characteristics, a higher prevalence of *Cryptosporidium* parasites was observed in clear water (Fig. 1).

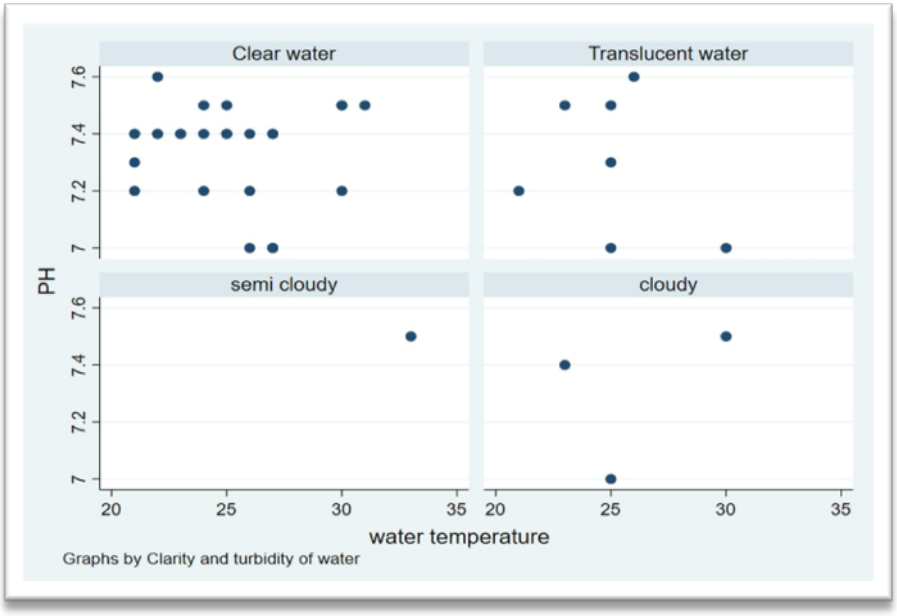


**Fig. 1:** Frequency of association between *Cryptosporidium*, physical characteristics (Clear, Translucent, Semi Cloudy, Cloudy water) of water that can affect the survival and presence of *Cryptosporidium*, water, temperature, and water pH in two Zahedan and Zabol Cities

This study examined the relationship between *Cryptosporidium* frequency and water clarity in each city. More parasites were found in the semi-cloudy water of Zabol and the cloudy water of Zahedan. *Cryptosporidium* was not detected in saline water.

Continuous surveillance and monitoring are crucial for identifying and responding to potential *Cryptosporidium* outbreaks in water, including routine testing of treated water (10).

## Conflict of Interest

The authors declare that there is no conflict of interests.

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