

Proceedings

Genotyping of *Cryptosporidium* spp. from a Sewage-Contaminated River in Guilan, Iran [†]

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Abstract: The role of water in *Cryptosporidium* transmission is now well recognized. In the present study, 19 water samples were collected from the river contaminated with wastewater in Guilan, Iran. This province lies along the Caspian Sea and has a Mediterranean-like climate. The species of *Cryptosporidium* were identified by polymerase chain reaction (PCR) assay using two set primers: (CPB-DIAGF/R and N-DIAGF2/R2) and (Xiao F1/R1 and F2/R2). A total of 10/19 water samples were positive for *Cryptosporidium* species (*C. parvum*, *C. muris* and *C. hominis*). The use of sewage-contaminated river water for the irrigation of agriculture farms is a threat of infection to the local population.

Keywords: *Cryptosporidium*; water; PCR; Iran

1. Introduction

Recent molecular studies of cryptosporidiosis in animals and the environment helped researchers to better understand the transmission of cryptosporidiosis in humans and the public health significance of *Cryptosporidium* spp. [1]. Waterborne cryptosporidiosis has been reported worldwide, and the ingestion of oocyst-contaminated drinking water has led to a large number of outbreaks, mainly reported from North America, the United Kingdom, Japan, and Australia [2–4]. The use of molecular techniques in epidemiological studies has shown that five *Cryptosporidium* species (*C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, and *C. canis*) are responsible for most human infections and two species, *C. parvum* and *C. hominis*, are the most common [5]. The distribution of *Cryptosporidium* species in environmental water and wastewater in Iran is unclear. The objective of this study was to identify the genotype of *Cryptosporidium* spp. in two important rivers in Iran.

2. Materials and Methods

2.1. Geography and Sample Collection

Guilan Province (37.2809° N, 49.5924° E) lies along the Caspian Sea and has a moderate, mild, and Mediterranean-like climate. Goharrood river is a branch of the Sefidrood River that crosses Rasht City and finally meets the Bandar-e Anzali Lagoon. In the present study, 19 water samples were collected from the Goharrood River. Many pollutants, such as rural, urban, and agricultural runoff and animals' raw sewage threaten water supplies in the cities of Guilan [6]. About 5 L of each

sample was filtered through a cellulose nitrate filter of pore size 1.2 µm using a vacuum pump. The sediment pellet was subjected to sucrose flotation [7], then the supernatant was subject to DNA extraction and Polymerase Chain Reaction (PCR).

2.2. DNA Extraction and PCR

DNA was extracted using the QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions with some modifications. Briefly, the suspension, including amoeba cysts or trophozoites, was frozen and thawed at −196 °C for 5 min ten times, followed by 5 min in boil water [8]. Two primer sets were used in this study. The first was a genus-specific primer targeting the 18 rRNA gene—specifically for *Cryptosporidium* spp.—used for the amplification of the approximately 435 bp fragment using CPB-DIAGF/R and N-DIAGF2/R2 primers [9,10].

The second set, XIAOF1/R1 and XIAO F2/R2, were used to amplify the approximately 850 bp fragment of the gene encoding the 18SSU rRNA of the genus *Cryptosporidium* as described previously [11,12]. Each reaction was performed in 50 µL and both PCR reactions were performed in PCR tubes according to a previous study [9–12]. The PCR products electrophoresis was performed on 1.5% gel agarose stained with ethidium bromide solution and visualized under UV light. The PCR products were then sequenced and all sequences data were manually edited and analyzed with reference sequences using the Chromas software program. Genotype identification was performed by the available *Cryptosporidium* DNA sequences in GenBank.

3. Results

In the present study, 10/19 of the samples were positive for *Cryptosporidium* spp. two nested PCR primers. DNA sequencing of five PCR products revealed the presence of three species of *Cryptosporidium*, *C. parvum* (3), *C. muris* (1) and *C. hominis* (1), respectively. *C. parvum* was the most common species.

4. Discussion

The detection of pathogens and the safety of drinking water are topics of great concern to health authorities. Accurate identification of *Cryptosporidium* species requires the use of genotyping tools. Currently, most *Cryptosporidium* genotyping tools use PCR targeting the small subunit (SSU) rRNA gene [13]. Molecular studies have shown that five *Cryptosporidium* species (*C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, and *C. canis*) are responsible for most human infections, but two species—*C. hominis* and *C. parvum*—are the most common, and the role of water in disease transmission is now well recognized [5]. Numerous outbreaks of human cryptosporidiosis all over the world have been reported, mainly from North America, the United Kingdom, Japan, and Australia [2–4,14]. In the present study, *C. parvum*, *C. muris* and *C. hominis* were detected. The occurrence of species other than *C. parvum* in the environment is well recognized. Xiao et al. [15,16] reported the presence of *Cryptosporidium* spp. in 27 of 29 storm water samples in the United States, mainly wildlife *Cryptosporidium* genotypes. In contrast, the most common species found in surface waters were *C. parvum*, *C. hominis*, and *C. andersoni*, with the last reported to be the most commonly found in wastewater (eight samples). Mahmoudi et al. [11,12] analyzed surface and river water samples in Iran by different methods, and showed that *C. parvum* was the most prevalent species, followed by *C. hominis* and *C. canis*, respectively [11,12]. Another study [17] reported this parasite from untreated water samples in Iran. In a comprehensive study by Keshavarz et al. (2009), Iranian cattle were mainly found to be infected with *C. parvum* (72.6%), *C. andersoni* (17.7%), and *C. bovis* (7.8%) [18]. *C. parvum* was the predominant species in children with diarrhea and AIDS patients in other studies in Iran [19,20]. Other studies in Iran have shown a frequent occurrence of *C. hominis* in children or AIDS patients [21,22]. *C. muris* was detected in the present study, which is a well-known rodent parasite [23]; the results of the present study indicate that these animals are also a source of *Cryptosporidium* oocysts in river water in Iran. The differences in the distribution of *Cryptosporidium*

species in humans are considered an indication of differences in infection sources [1]. *C. hominis* is responsible for far more infections than *C. parvum* in humans in developing countries, where genotyping studies have been conducted [24–29]. In the United Kingdom, Europe, and New Zealand, *C. parvum* was as common as *C. hominis* in humans [30–34]. The results of the present study indicate that the investigated river water supplies were contaminated by pathogenic species of *Cryptosporidium* from humans and animals.

5. Conclusions

The use of long-term sewage-contaminated river water for the irrigation of crops and agriculture farms and their subsequent transfer to the food chain is a threat of infection to the population. More future studies with a systematic sampling of water in the study area would provide a better picture of the extent of water contamination with *Cryptosporidium* species. Periodic determination of *Cryptosporidium* spp. in river water can help to develop strategies for the protection of human health.

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