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Original Article

Molecular Epidemiology of Cryptosporidiosis in Iranian Children, Tehran, Iran

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ABSTRACT

Background: *Cryptosporidium* is a worldwide protozoan parasite and one of the most common causes of infection and diarrhea in humans and cattle. The aim of the present study was determination of subtypes of *Cryptosporidium* among children with diarrhea in Tehran by sequence analysis of the highly polymorphic 60-kDa glycoprotein (*GP60*) gene.

Methods: Fecal samples were collected from 794 diarrheic children. Initial identification of *Cryptosporidium* was carried out on stool samples by Ziehl-Neelsen acid-fast staining method. DNA was extracted from positive microscopically samples and *Cryptosporidium* genotypes and subtypes were determined, accordingly.

Results: Out of 794 collected samples, 19 (2.40 %) were positive for *Cryptosporidium* oocysts. Sequences analysis of *GP60* gene showed that 17 (89.47 %) of the positive isolates were *Cryptosporidium parvum* and 2 (10.52 %) were *C. hominis*. All subtypes of *C. parvum* isolates belonged to allele families IIa (6/17) and IIc (11/17). The most common allele in all 17 isolates belonged to IIc A20G1a (41.18%). A22G1 (IF) subtype was detected in two *C. hominis* isolates of the children.

Conclusion: The predominancy of *C. parvum* species (specially, IIc A20G1a subtype) in current study underlines the importance of zoonotic *Cryptosporidium* transmission in Iran.

Keywords: Genotypes, Subtypes, *Cryptosporidium*, GP60 gene, Children, Iran

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Introduction

Cryptosporidiosis is a frequent cause of diarrheal diseases in humans. Molecular tools have generally been applied for the identification of the *Cryptosporidium* isolates from humans and animals at the species or genotype and subtype levels (1, 2). DNA sequencing analysis of the *Cryptosporidium* 60-kDa glycoprotein (*GP60*) gene has shown comprehensive genetic heterogeneity among *C. hominis*, *C. parvum*, and *C. meleagridis* isolates. Several subtype groups have been identified in these species, accordingly: 7 subtype groups in *C. hominis* (Ia–Ig), 2 zoonotic (IIa, IIc) and 10 non-zoonotic subtype groups (IIb, IIe–III) in *C. parvum*, and 6 subtype groups in *C. meleagridis* (3–9). Within each subtype groups, there are several subtypes primarily based on the number of tri-nucleotide repeats coding for the amino acid serine (6).

There are several molecular studies, which have documented the prevalence of *C. parvum* and *C. hominis* in Iran (10–13). At present study, we have identified the genotypes and subtypes of the *Cryptosporidium* isolates from Iranian children with diarrhea using polymerase chain reaction (PCR) and DNA sequencing analysis of the *GP60* gene to determine their prevalence among endemic species of *Cryptosporidium* in Tehran province, Iran.

Materials and Methods

Samples

From December 2005 to September 2007, total of 794 fecal samples were collected from children with diarrhea whom referred to Pediatrics Hospital in Tehran, Iran. *Cryptosporidium* oocysts were detected in samples which concentrated by formalin-ethyl-acetate sedimentation and stained with

a modified Ziehl-Neelsen technique (13). The positive *Cryptosporidium* spp. samples were preserved in 2.5% potassium dichromate and kept at 4°C until DNA extraction.

DNA extraction

About 300 µl of fecal suspension was washed three times with distilled water to remove trace of dichromate and the genomic DNA was extracted by DNAzol kit according to the manufacturer's instructions (Invitrogen, life technologies, Cat. No 10503-027, USA) with addition of three times 10 min freeze-thaw cycles after resuspending in lysis buffer in order to rupture the *Cryptosporidium* oocysts.

GP60 PCR- Sequencing

PCR kit (Ampliqon ApS, literbuen 11, DK-2740 Skovlunde, Denmark) was used to accelerate and increase the quality of PCR products. Nested *GP60* PCR was performed according to Alves et al. (4). Primary amplification was carried out in 32 cycles of 94°C for 45 s, 40°C for 45 s, and 72°C for 1 min, with an initial denaturation at 94°C for 3 min and a final extension at 72°C for 7 min. For secondary amplification, 32 cycles of 94°C for 35 s, 40°C for 35 s, and 72°C for 1 min were used, with identical initial denaturation and final extension conditions. Amplified fragments have been purified in Centri-Sep spin columns (Princeton Separations, New Jersey, USA) and sequenced in a 3100ABI PRISM Genetic Analyzer (Applied Biosystems, NY, USA).

Results

Out of 794 collected samples, *Cryptosporidium* oocysts were found in 19 (2.40%). The PCR products of *GP60* gene were purified and sequenced using genetic analyzer ma-

chine (Fig. 1). The sequence analysis of *GP60* gene showed that 17 (89.47%) and 2 (10.52%) of these isolates belonged to *C. parvum* and *C. hominis*, respectively. The sequences were determined and analysed using the chromas program and aligned with each other and with previously reported sequences for identification of the alleles and subtypes.

The result of this analysis showed that all subtypes of *C. parvum* isolates belonged to

the families IId (11/17) and IIa (6/17), while Subtype family If (If A22G1) was just observed in two *C. hominis* isolates from children. Three subtypes within the subtype family IId including IId A18G1 (3/17), IId A20G1a (7/17) and IId A15G1 (1/17) were recognized and one subtype within the subtype family IIa (IIa A15G2R1 (6/17)) was identified as well (Table 1).

Table 1: Distribution of *Cryptosporidium* genotypes and subtypes in isolates from Iranian children with diarrhea

| Subtype | No. of isolates | Species | Source | Accession number |
|-------------|-----------------|-------------------|----------|------------------|
| IIa A15G2R1 | 6 | <i>C. parvum</i> | children | AB560747 |
| IId A18G1 | 3 | <i>C. parvum</i> | children | AB560743 |
| IId A20G1a | 7 | <i>C. parvum</i> | children | AB560745 |
| IId A15G1 | 1 | <i>C. parvum</i> | children | AB560748 |
| If A22G1 | 2 | <i>C. hominis</i> | children | AB560750 |

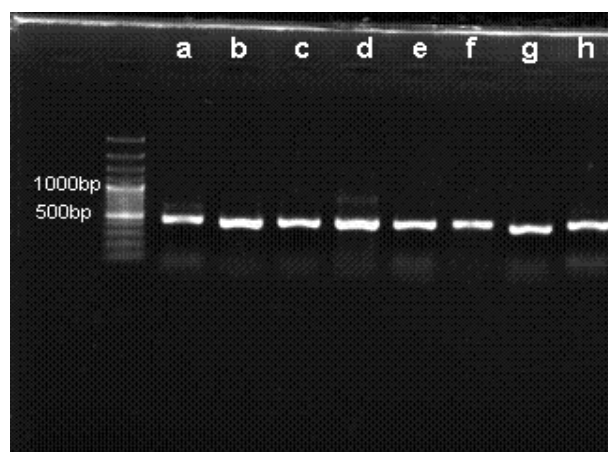


Fig. 1: PCR of *Cryptosporidium* based on *GP60* gene. Lane 1: 100 bp DNA marker, lane a-h: *C. parvum* and *C. hominis*

Discussion

In the developing countries, the association of *Cryptosporidium* with acute and persistent diarrhea in children is well recognized. Ac-

ording to the previous studies (based on microscopic examination of fecal samples), the prevalence of this parasite in various parts of

Iran was 4.1% in west, 7% in southeastern, 2.2% in south, and 7.7% in northwest (13). In the present study, the prevalence of *Cryptosporidium* in children was estimated 2.4%.

The prevalence of *Cryptosporidium* species among children in different regions is varied. Studies conducted in Kenya, Thailand, Peru, South India, Malawi and South Africa have shown *C. hominis* as a predominate species in children or HIV positive adults (3, 5, 14-17), while in our study the sequence analysis of *GP60* gene indicated that *C. parvum* with a rate of 89.47% was the predominant species in children. This finding has had a close agreement with the previous molecular studies in Iran (10-13) as well as other studies performed in UK, Kuwait, Portugal, France, North America, and the Netherland (4, 6, 18-21). Among the allele families identified in Iranian children, the most common subtype families were IIa and IIc, which was in consistent with results from Kuwait (6). There are studies conducted in South Africa, Malawi, Portugal, Northern Ireland, and the United States, in which positive samples were more or less evenly distributed among the common allele families Ia, Ib, Id, Ie, IIa, and IIc (1,2, 4, 5, 8, 9,18, 21).

The frequent subtype in subtype families IIc among the isolates of Iranian children was IIc A20G1. Amer et al. documented the presence of the predominant *C. parvum* subtype IIc A20G1 in children, whereas this subtype was previously seen as main subtype in Egyptian cattle (22). Also the other human subtypes IIc A15G1 and IIc A18G1 similarly had been identified in Spanish lambs and goat (23). According to our results, neither *C. hominis* Ib subtype family, commonly seen in Australia and European infected human (20) nor *C. hominis* IIc subtype family, commonly seen in Peru infected children (16), were seen in Iranian infected individuals. In this study, the rare subtype

family If (If A22G1) was also seen in two Iranian children.

The limitation of this study was the lack of appropriate epidemiological data for compares the analysis of *Cryptosporidium* subtypes. According to the defined zoonotic patterns of the isolates in this study, it seems that direct or indirect contact with animals is the main mode of cryptosporidiosis transmission in Iran. Further molecular characterization on human and animals is needed to increase our knowledge about *Cryptosporidium* transmission and its epidemiology.

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The authors declare that there is no conflict of interests.

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