Molecular characterization of Cryptosporidium spp. and Giardia duodenalis in children in Egypt

Doaa Naguib1,2, Adel H. El-Gohary1, Dawn Roellig2, Amro A. Mohamed1, Nagah Arafat3, Yuanfei Wang2,4, Yaoyu Feng4 and Lihua Xiao4*

Abstract

Background: The transmission of Cryptosporidium spp. and Giardia duodenalis into humans varies according to species/genotypes of the pathogens. Although infections with both parasites are recorded in Egypt, few data are available on the distribution of Cryptosporidium species and G. duodenalis genotypes. The present study assessed the occurrence and genetic diversity of Cryptosporidium spp. and G. duodenalis in Egyptian children.

Methods: In the present study, 585 fecal specimens were collected from children eight years old and younger in three provinces (El-Dakahlia, El-Gharbia and Damietta) during March 2015 to April 2016. PCR-RFLP analysis of the small subunit rRNA gene and sequence analysis of the 60 kDa glycoprotein gene were used to detect and subtype Cryptosporidium spp., respectively, whereas PCR and sequence analyses of the triose phosphate isomerase, glutamate dehydrogenase and β-giardin genes were used to detect and genotype G. duodenalis.

Results: The overall infection rates of Cryptosporidium spp. and G. duodenalis were 1.4% and 11.3%, respectively. The Cryptosporidium species identified included C. hominis and C. parvum, each with three subtype families. The C. hominis subtypes were IbA6G3 (n = 2), IdA17 (n = 1), IdA24 (n = 1) and IfA14G1R5 (n = 1), while C. parvum subtypes were IIdA20G1 (n = 1), IlaA15G2R1 (n = 1), and IlcA5G3a (n = 1). The G. duodenalis identified included both assemblages A (n = 31) and B (n = 34). All G. duodenalis assemblage A belonged to the anthroponotic sub-assemblage AII, while a high genetic heterogeneity was seen within assemblage B.

Conclusions: Data from this study are useful in our understanding of the genetic diversity of Cryptosporidium spp. and G. duodenalis in Egypt and the potential importance of anthroponotic transmission in the epidemiology of both pathogens.

Keywords: Cryptosporidium, Giardia duodenalis, Children, Egypt, Epidemiology, Subtypes

Background

Diarrhea is a worldwide public health issue, responsible for 2.3 billion sicknesses and 1.3 million deaths in 2015. It is the second most important cause of death among children under 5 years of age [1]. Most of the deaths are recorded in developing countries, particularly African countries. Various gastrointestinal pathogens, including bacteria, viruses and parasites cause diarrhea. Among the latter, Cryptosporidium spp. and Giardia duodenalis are common etiological agents in humans and animals globally [2, 3]. Cryptosporidium is second only to rotavirus in causing diarrhea and death in children in developing countries, responsible for 2.9 million cases annually in children aged < 24 months in the sub-Saharan Africa [4, 5]. Similarly, G. duodenalis is responsible for ~280 million cases of intestinal diseases per year worldwide [6]. Cryptosporidium spp. and G. duodenalis are transmitted in humans through the fecal-oral route, either directly by person-to-person transmission or contact with infected animals or indirectly via food-borne or water-borne transmission following ingestion of contaminated food or water [2, 3].
Currently, over 30 Cryptosporidium species have been recognized, but humans are mostly infected with C. parvum and C. hominis [7] with the former mostly transmitted anthroponotically while the latter can be transmitted either anthroponotically or zoonotically [8]. Similarly, among the eight established G. duodenalis genotypes (frequently referred as assemblages) identified using molecular tools, assemblages A and B are responsible for most human infections. Between them, assemblage A is also commonly seen in animals and thus could be responsible for zoonotic G. duodenalis infection [8, 9].

It has been noted that some subtype families of C. parvum are more frequently found in certain host species, such as IIa in cattle, IIc in humans, and IId in sheep and goats. While all three subtype families of C. parvum can infect humans, their distribution in humans differs geographically and socioeconomically, probably as a result of differences in the importance of various transmission routes [8]. Similarly, host adaptation also occurs within G. duodenalis assemblage A, with AI subtypes being more commonly found in domestic animals, AI subtypes mostly in humans, and AIII subtypes almost exclusively in wild ruminants [8, 9]. Thus, molecular characteristics of Cryptosporidium spp. and G. duodenalis at species and subtype levels are helpful in improving our understanding of cryptosporidiosis and giardiasis epidemiology [7].

Compared with other countries, few data exist on the occurrence of Cryptosporidium and G. duodenalis genotypes and subtypes in humans in Egypt. Previous microscopic and serologic studies had shown a common occurrence of Cryptosporidium spp. and G. duodenalis in humans in the country [10–12]. Only a few studies have examined the molecular characteristics of Cryptosporidium spp. and G. duodenalis in a small number of human clinical specimens [13–18]. The current study was conducted to collect data on the distribution of Cryptosporidium and G. duodenalis genotypes and subtypes in kindergarten age children (≤ 8 years) in order to improve our understanding of the transmission of these parasites in Egypt.

**Methods**

**Specimen collection**

This study was conducted during March 2015 to April 2016 in El-Dakahlia, El-Gharbia, and Damietta provinces, Egypt (Fig. 1). Fresh stool specimens were collected monthly from 585 different children in 18 childcare centers, who ranged 2 to 8 years in age (median age: 4 years). These specimens were collected individually in sterile plastic cups and transported to the laboratory in coolers. Information on the age, gender, diarrhea and health status, animal contact and residency, was recorded from parents or guardians. Specimens were preserved in 70% ethanol and kept at 4 °C to prevent DNA deterioration prior to DNA extraction at the Centers for Disease Control and Prevention, Atlanta, GA, USA. No microscopy of pathogens was conducted during the study. Informed consent was obtained from the parents or guardians of the study children.

**DNA extraction**

Stored stool specimens were washed twice with distilled water by centrifugation to remove ethanol. DNA was extracted from washed fecal materials using the FastDNA SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA) and manufacturer-recommended procedures. DNA was eluted in 100 μl molecular grade water and stored at -20 °C prior to molecular analyses.

**Cryptosporidium detection, genotyping and subtyping**

All specimens were examined for Cryptosporidium spp. using a nested polymerase chain reaction (PCR) targeting a ~834 bp fragment of the small subunit rRNA (SSU rRNA) gene [19]. C. parvum- and C. hominis-positive specimens were further analyzed by a nested PCR targeting a ~850 bp fragment of the 60 kDa glycoprotein (gp60) gene [20]. Each analysis was conducted in duplicate, using C. baileyi and C. parvum DNA as the positive control for SSU rRNA and gp60 PCR, respectively, and reagent-grade water as the negative control. Cryptosporidium species in the positive specimens were identified by RFLP analysis of the secondary SSU rRNA PCR products using restriction enzymes SspI (New England BioLabs, Ipswich, MA, USA) and VspI (Promega, Madison, WI, USA) as described [19]. C. hominis and C. parvum subtypes were identified by bidirectional DNA sequence analysis of the secondary PCR products of the gp60 gene [20].

**Giardia detection, genotyping and subtyping**

All 585 specimens were analyzed for G. duodenalis using nested-PCR assays targeting 3 genetic loci, including triose phosphate isomerase (tpi) [21], beta-giardin (bg) [22] and glutamate dehydrogenase (gdh) [23] genes. Specimens were identified as G. duodenalis-positive when the expected PCR product was obtained from at minimum one of the three loci. G. duodenalis genotypes and subtypes were identified by bidirectional DNA sequence analysis of the secondary PCR products.
ChromasPro software (www.technelysium.com.au/ChromasPro.html). They were aligned against each other and reference sequences [7, 9] using ClustalX software (http://www.clustal.org/) to identify Cryptosporidium subtypes and G. duodenalis assemblages and subtypes. Multilocus genotypes (MLGs) of G. duodenalis assemblage A were identified based on nucleotide sequences at the tpi, bg, and gdh loci, using the established nomenclature system [9].

Statistical analysis
The Chi-square test was used to compare Cryptosporidium and G. duodenalis infection rates between age groups (≤ 3 to 8 years), gender (boys and girls), residency (urban and rural), and children with and without gastrointestinal symptoms (diarrhea and abdominal pain) or animal contact (with and without). The relationship between age and diarrhea was assessed using the nonparametric Kendall’s tau_b and Spearman’s rho tests. The statistical analysis was performed using the SPSS software version 20.0 (IBM, Armonk, NY, USA). Differences were considered significant at \( P < 0.05 \).

Results
Occurrence of Cryptosporidium spp. and G. duodenalis
Of the 585 fecal specimens examined in this study from kindergarten children, 8 (1.4%) and 66 (11.3%) were positive for Cryptosporidium spp. and G. duodenalis, respectively. No concurrence of the two pathogens was detected in any of the specimens.

By age, the highest rates of Cryptosporidium (2.7%) and G. duodenalis (14.2%) infections were detected in children of age ≤ 3 years and 4 years, respectively; neither Cryptosporidium nor G. duodenalis were detected in children of 8 years in age (Table 1). The infection rates of both protozoans were similar between girls and boys (1.0% and 1.7% for Cryptosporidium and 11.1% and 11.5% for G. duodenalis, respectively) \( (\chi^2 = 0.460, P = 0.49 \text{ and } \chi^2 = 0.011, P = 0.91, \text{ respectively}) \).

Cryptosporidium infection rate was 2.3% and 1.2 % in children with and without diarrhea, respectively \( (\chi^2 = 0.576, P = 0.44) \). In contrast, the infection rate of G. duodenalis was significantly higher in diarrheic children (19.1%) than in non-diarrheic ones (9.9%) \( (\chi^2 = 6.149, P = 0.01) \). There was also an insignificantly higher occurrence of Cryptosporidium spp. in children with
abdominal pain (2.0%) than those without it (0.4%) ($\chi^2 = 2.612, P = 0.10$). In contrast, *G. duodenalis* infection rates were similar between the two groups (10.8% and 12.0%, respectively; $\chi^2 = 0.134, P = 0.71$). The infection rates of *Cryptosporidium* and *G. duodenalis* were similar between children with (1.2% and 10.5%, respectively) and without (1.5% and 11.9%, respectively) animal contact ($\chi^2 = 0.146, P = 0.92$ and $\chi^2 = 0.128, P = 0.93$, respectively). In addition, children in rural areas had *Cryptosporidium* and *G. duodenalis* infection rates (1.5% and 12.1%, respectively) similar to those in urban areas (1.2% and 10.3%, respectively; $\chi^2 = 0.091, P = 0.76$ and $\chi^2 = 0.339, P = 0.56$, respectively; Table 1). The infection rate of *Cryptosporidium* spp. in El-Dakahlia (1.8%) was higher than in El-Gharbia (1.1%) and Damietta (0.8%). In contrast, the infection rate of *G. duodenalis* was higher in El-Dakahlia (11.4%) and El-Gharbia (12.7%) than in Damietta (8.9%; Table 1).

There was a significant negative correlation between age and diarrhea (correlation coefficient was -0.115 and -0.127. by Kendall’s tau_b and Spearman’s rho tests, respectively; $P = 0.002$ in both tests).

### Cryptosporidium species and subtypes

The RFLP analysis of the SSU rRNA PCR products identified the presence of *C. hominis* in five specimens and *C. parvum* in three specimens (Table 2). Three subtype families were identified within *C. hominis* and *C. parvum* each by gp60 sequence analysis. The *C. hominis* subtypes families included Ib (in two specimens), Id (in

| Table 1 | Occurrence of *Cryptosporidium* spp. and *Giardia duodenalis* in children by age, gender, diarrhea or abdominal pain occurrence, animal contact, residency and locality |
|---------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Variable | No. of samples | No. of positive (%) | *Cryptosporidium* spp. 95% confidence interval | *Giardia duodenalis* 95% confidence interval | | |
| | | | Lower limit | Upper limit | Lower limit | Upper limit | Lower limit | Upper limit |
| Age | | | | | | | |
| ≤ 3 years | 74 | 2 (2.7) | -0.009 | 0.063 | 7 (9.5) | 0.028 | 0.161 |
| 4 years | 141 | 3 (2.1) | -0.002 | 0.044 | 7 (14.2) | 0.084 | 0.199 |
| 5 years | 190 | 1 (0.5) | -0.005 | 0.015 | 26 (13.7) | 0.088 | 0.185 |
| 6 years | 136 | 2 (1.5) | -0.005 | 0.035 | 10 (7.4) | 0.030 | 0.117 |
| 7 years | 27 | 0 (0.0) | 0.000 | 0.000 | 3 (11.1) | -0.007 | 0.229 |
| 8 years | 17 | 0 (0.0) | 0.000 | 0.000 | 0 (0.0) | 0.000 | 0.000 |
| Gender | | | | | | | |
| Female | 289 | 3 (1.0) | -0.001 | 0.021 | 32 (11.1) | 0.074 | 0.147 |
| Male | 296 | 5 (1.7) | 0.002 | 0.031 | 34 (11.5) | 0.078 | 0.151 |
| Diarrhea occurrence | | | | | | | |
| Yes | 89 | 2 (2.3) | -0.008 | 0.054 | 17 (19.1)$^a$ | 0.109 | 0.272 |
| No | 496 | 6 (1.2) | 0.002 | 0.021 | 49 (9.9)$^a$ | 0.72 | 0.125 |
| Abdominal pain | | | | | | | |
| Yes | 351 | 7 (2.0) | 0.005 | 0.034 | 38 (10.8) | 0.075 | 0.140 |
| No | 234 | 1 (0.4) | -0.004 | 0.012 | 28 (12.0) | 0.078 | 0.161 |
| Animal contact | | | | | | | |
| With | 257 | 3 (1.2) | -0.001 | 0.025 | 27 (10.5) | 0.067 | 0.142 |
| Without | 328 | 5 (1.5) | 0.001 | 0.028 | 39 (11.9) | 0.083 | 0.154 |
| Residency | | | | | | | |
| Rural | 332 | 5 (1.5) | 0.001 | 0.028 | 40 (12.1) | 0.085 | 0.156 |
| Urban | 253 | 3 (1.2) | -0.001 | 0.025 | 26 (10.3) | 0.065 | 0.140 |
| Locality | | | | | | | |
| El-Dakahlia | 272 | 5 (1.8) | 0.002 | 0.033 | 31 (11.4) | 0.076 | 0.151 |
| El-Gharbia | 189 | 2 (1.1) | -0.003 | 0.025 | 24 (12.7) | 0.079 | 0.174 |
| Damietta | 124 | 1 (0.8) | -0.007 | 0.023 | 11 (8.9) | 0.038 | 0.139 |

*The difference between the two groups is significant*
two specimens) and If (in one specimen), while the *C. parvum* subtypes families included IIa, IIc, and IId (in one specimen each). There were two subtypes (IdA17 and IdA24) in the subtype family IId and one subtype each in subtype families Ib (IbA6G3 in two specimens) and If (IfA14G1R5 in one specimen). The *C. parvum* subtypes detected included IIaA15G2R1, IIdA20G1 and IIcA5G3a (in one specimen each).

**Giardia duodenalis** genotypes and subtypes

Of the 66 *G. duodenalis*-positive specimens, 56 were positive in *tpi* PCR, 48 in *gdh* PCR, and 55 in *bg* PCR. Among them, 31 (47.0%) had assemblage A and 34 (51.5%) had assemblage B, with one specimen (1.5%) being positive for both assemblages A and B (Table 3). The latter was indicated by the identification of assemblage B at the *tpi* and *gdh* loci but assemblage A at the *bg* locus. There were mostly no double peaks in the chromatograms generated from the study. Assemblage A was identified in 28 specimens based on *tpi* and *bg* sequence analyses but in 25 specimens by *gdh* sequence analysis. In contrast, assemblage B was found in 28, 23 and 27 specimens at the *tpi*, *gdh* and *bg* loci, respectively (Table 3). The relative distribution of *G. duodenalis*

### Table 2 Characteristics of eight Cryptosporidium-positive children

| Cryptosporidium spp. | Subtypes | Age (years) | Gender | Diarrhea occurrence | Abdominal pain occurrence | Animal contact | Residency |
|----------------------|----------|-------------|--------|---------------------|---------------------------|---------------|-----------|
| *Cryptosporidium hominis* | IbA6G3<sup>a</sup> | 4 | Female | No | Yes | No | Urban |
| *Cryptosporidium hominis* | IbA6G3<sup>a</sup> | 4 | Male | No | Yes | No | Urban |
| IdA17 | 2 | Male | No | Yes | No | Urban |
| IdA24 | 5 | Male | No | Yes | No | Rural |
| IfA14G1R5 | 3.5 | Female | No | No | No | Rural |
| *Cryptosporidium parvum* | IIA15G2R1 | 5.5 | Male | Yes | Yes | Yes | Rural |
| *Cryptosporidium parvum* | IIdA20G1<sup>a</sup> | 3 | Male | Yes | Yes | Yes | Rural |
| IIC5G3a | 6 | Female | No | Yes | Yes | Rural |

<sup>a</sup>New subtype identified in humans in Egypt.

### Table 3 Distribution of *G. duodenalis* assemblages in children from different kindergartens at the *tpi*, *gdh* and *bg* loci

| Study area<sup>a</sup> | No. of samples | No. of positive (%) | Number of positive |
|------------------------|----------------|---------------------|--------------------|
|                        |                |                     | *tpi*              |
|                        |                |                     | Assemblage A | Assemblage B | Assemblage A | Assemblage B | Assemblage A | Assemblage B |
| El-Dakahlia            |                |                     |                   |               |               |               |               |               |
| K1                     | 34             | 2 (5.9)             | 0                 | 2             | 0             | 1             | 0             | 1             |
| K2                     | 39             | 8 (20.5)            | 5                 | 3             | 3             | 3             | 4             | 3             |
| K3                     | 33             | 3 (9.1)             | 2                 | 0             | 3             | 0             | 2             | 0             |
| K4                     | 31             | 3 (9.7)             | 1                 | 2             | 1             | 2             | 1             | 2             |
| K5                     | 22             | 2 (9.1)             | 2                 | 0             | 1             | 0             | 1             | 0             |
| K6                     | 36             | 6 (17.1)            | 2                 | 4             | 2             | 3             | 3             | 2             |
| K7                     | 37             | 2 (5.4)             | 0                 | 2             | 0             | 2             | 0             | 2             |
| K8                     | 41             | 5 (12.2)            | 1                 | 3             | 1             | 3             | 1             | 4             |
| El-Gharbia             |                |                     |                   |               |               |               |               |               |
| K1                     | 27             | 3 (11.1)            | 2                 | 1             | 1             | 1             | 1             | 1             |
| K2                     | 30             | 5 (16.7)            | 2                 | 2             | 2             | 2             | 2             | 3             |
| K3                     | 34             | 5 (14.7)            | 4                 | 1             | 3             | 1             | 4             | 1             |
| K4                     | 29             | 2 (6.9)             | 0                 | 2             | 0             | 1             | 0             | 1             |
| K5                     | 32             | 4 (12.5)            | 1                 | 2             | 1             | 0             | 1             | 2             |
| K6                     | 37             | 5 (13.5)            | 2                 | 2             | 2             | 1             | 2             | 2             |
| Darmietta              |                |                     |                   |               |               |               |               |               |
| K1                     | 22             | 3 (13.6)            | 1                 | 0             | 2             | 1             | 2             | 1             |
| K2                     | 28             | 4 (14.3)            | 1                 | 1             | 1             | 2             | 2             | 1             |
| K3                     | 38             | 4 (10.5)            | 2                 | 1             | 2             | 0             | 2             | 1             |
| K4                     | 36             | 0 (0.0)             | 0                 | 0             | 0             | 0             | 0             | 0             |
| Total                  | 585            | 66 (11.3)           | 28                | 28            | 25            | 23            | 28            | 27            |

<sup>a</sup>K, kindergarten
assemblages A and B was similar among three provinces (Table 4); assemblage A was detected in 14, 11 and 6 specimens from El-Dakahlia, El-Gharbia and Damietta provinces, respectively, whereas, assemblage B was detected in 16, 13 and 5 specimens, respectively.

**Multilocus genotypes (MLGs) of G. duodenalis**

Sequence analysis of the three genetic loci showed only limited genetic diversity in assemblage A. All identified subtypes were belonged to sub-assemblage AII. Therefore, at the *tpi* locus, all assemblage A sequences were identical to the A2 subtype sequence (U57897) in GenBank (Table 5). Similarly, at the *gdh* locus, all 25 assemblage A sequences were identical to the A2 subtype sequence (AY178737) in GenBank, while at the *bg* locus, 22 were identical to the A3 subtype (AY072724), 4 were identical to the A2 subtype (AY072723), and 2 belonged to a new subtype A9 (MG746615). Among the assemblage A specimens, 4 and 18 specimens had MLGs identical to the A2 subtype (AY072723), and 2 identified in only one specimen.

Much higher genetic diversity was seen in assemblage B (Additional file 1: Table S1). Of the 28 specimens that were positive for assemblage B at the *tpi* locus, 14 had generated sequences identical to either KX668322 (*n* = 3), JF918523 (*n* = 2), KT948107 (*n* = 2), KT948111 (*n* = 2), AB781127 (*n* = 1), KX468984 (*n* = 1), KY696816 (*n* = 1) or KX468984 (*n* = 1), while 14 specimens generated sequences of one of the 10 new types (MG787950–MG787959). Similarly, of the 23 specimens that were positive for assemblage B at the *gdh* locus, 14 had sequences identical to either KY696804 (*n* = 4), KM190714 (*n* = 3), KP687771 (*n* = 3), U362955 (*n* = 2), EF507654 (*n* = 1) or KP687770 (*n* = 1), while the remaining nine specimens produced sequences of one of the eight new types (MG746604–MG746611). At the *bg* locus, 24 specimens generated sequences identical to either KU504732 (*n* = 6), KY696836 (*n* = 5), JF918485 (*n* = 3), KU504720 (*n* = 2), KU504707 (*n* = 2), MF169196 (*n* = 2), AB480877 (*n* = 1), KT948086 (*n* = 1), KU504731 (*n* = 1) or KY483962 (*n* = 1), whereas three specimens yielded sequences that belonged to one of the three new subtypes (MG746612–MG746614). Altogether, 44 specimens were successfully subtyped at all three genetic loci, forming 3 MLGs of assemblage A and 20 MLGs of assemblage B.

**Discussion**

In the present study, the overall infection rates of *Cryptosporidium* spp. and *G. duodenalis* in children were 1.4 and 11.3%, respectively. Earlier studies based on microscopy had recorded 5.6–60.2% and 17.6–25.0% infection rates of *Cryptosporidium* spp. and *G. duodenalis* in Egyptian children, respectively [24–27]. A previous molecular analysis of fecal specimens from Egyptian children produced 49.1% and 21% infection rate for *Cryptosporidium* spp. and *G. duodenalis*, respectively [13, 17]. In the neighboring Lebanon, infection rates of 10.4% and 28.5% were reported in school children for *Cryptosporidium* spp. and *G. duodenalis*, respectively [28]. Similar low *Cryptosporidium* occurrence (1.6–2.0%) was observed in children in China [29, 30]. The low occurrence of *Cryptosporidium* spp. in this study might be due to the older age of children enrolled in this study. In developing countries, children under two years have the highest occurrence of *Cryptosporidium* spp. [4, 31]. In addition, children participating in the study were healthy kindergartners rather than in-patients and out-patients in most previous studies. As expected, children with diarrhea had higher occurrence of both *Cryptosporidium* spp. and *G. duodenalis* in this and earlier studies [28]. These are also supported by results of the nonparametric analysis of the negative correlation between age and occurrence diarrhea in this study.

In our study, we identified only *C. hominis* and *C. parvum* in children. This is similar to results of other studies in Egypt [13, 14, 32]. Moreover, the more common occurrence of *C. hominis* in children in this and other African studies suggests that anthroponotic transmission is important in cryptosporidiosis epidemiology in this region.
area, although the occurrence of zoonotic infections could not be fully excluded [13–15, 28, 32–35]. This is also supported by the identification of IIcA5G3a in C. parvum, which is considered a human-adapted C. parvum subtype [8]. In contrast, previous studies in the neighboring Mideast countries had shown a dominance of the zoonotic IIa and IId subtypes of C. parvum in children, which were only identified in two of the eight cryptosporidiosis cases in this study [36–40]. The insignificant associations between cryptosporidiosis occurrence and animal contact or rural residency in this study also support the importance of anthroponotic transmission in Cryptosporidium spp. in Egyptian children.

Although Cryptosporidium spp. were detected in only a few specimens in the study, we recorded seven subtypes in six families, including Ib, Id and If subtype families of C. hominis and IIa, IIc, and IId subtype families of C. parvum. This indicates that the transmission of Cryptosporidium in the study area is intensive. It has been reported that subtype families Ia, Ib, Id and Ie are common in children in developing countries [8, 31]. Nevertheless, the IbA6G3, IdA17, IdA24, and IfA14G1R5 identified in this study are rare subtypes within these common C. hominis subtype families [8, 31], indicating that C. hominis transmission in Egypt is probably autochthonous in nature.

The genotypes (assemblages of similar sequence types identified by multilocus molecular characterization) of G. duodenalis in infected children from the three provinces in this study belonged to assemblages A and B. This agrees with the findings of a recent study of G. duodenalis in children in Egypt [18]. The assemblages E and C reported in a few Egyptian children in previous studies [16, 17] were not detected in the present study. The equal occurrence of assemblages A and B in the present study is in discordance with observations in previous Egyptian studies, which showed a dominance of assemblage B in children [16–18]. Globally, assemblage B is more common than assemblage A in humans [7]. As assemblage B is much less frequently detected in animals [2], G. duodenalis transmission in Egyptian children appears to be mostly anthroponotic. This is also supported by the identification of assemblage A isolates in the study as the sub-assemblage AII, which is preferentially found in humans [7].

In this study, a much higher genetic diversity was observed in assemblage B than in assemblage A. Similar observations were made in previous studies [2]. This could be due to the more frequent occurrence of genetic recombination among assemblage A isolates, as assemblage B is known to have much higher allelic sequence heterozygosity than assemblage A. The existence of highly genetic variations among isolates of assemblage B has led to the inability of categorizing assemblage B isolates into well-defined specific sub-assemblages [9]. Comparative genomics rather than current MLG analysis might be needed for better characterization of assemblage B isolates [41].

**Conclusions**

Giardiasis is apparently common, and cryptosporidiosis remains to be a problem in kindergarten age children in Egypt. The dominance of C. hominis and common occurrence of G. duodenalis assemblage B and sub-assemblage AII in clinical specimens showcases the important role of anthroponotic transmission in disease epidemiology, although the occurrence of zoonotic infections could not be totally ruled out. Improved sanitation and hygiene and other intervention measures such as better health communication and the provision of clean and safe drinking water should be implemented to reduce the occurrence of cryptosporidiosis and giardiasis and minimize the impact of diarrhea on pediatric health in the country.
25. Abdel-Hafeez EH, Ahmad AK, Ali BA, Moslam FA. Opportunistic parasites among immunosuppressed children in Minia District, Egypt. Korean J Parasitol. 2012;50:57–62.

26. Abdel-Messih IA, Wierzba TF, Abu-Elyazeed R, Ibrahim AF, Ahmed SF, Kamal K, et al. DIarrhea associated with Cryptosporidium parvum among young children of the Nile River delta in Egypt. J Trop Pediatr. 2005;51:154–9.

27. El-Mohamady H, Abdel-Messih IA, Yousef FG, Said M, Farag H, Shaheen HI, et al. Enteric pathogens associated with diarrhea in children in Fayoum, Egypt. Diagn Microbiol Infect Dis. 2006;56:1–5.

28. Osman M, El Safadi D, Cian A, Benamrouz S, Nourrisson C, Poinier P, et al. Prevalence and risk factors for intestinal protozoan infections with Cryptosporidium, Giardia, Blastocystis and Dientamoeba among schoolchildren in Tripoli, Lebanon. PLoS Negl Trop Dis. 2016;10:e0004496.

29. Feng Y, Wang L, Duain L, Gomez-Puerta LA, Zhang L, Zhao X, et al. Extended outbreak of cryptosporidiosis in a pediatric hospital, China. Emerg Infect Dis. 2012;18:312–4.

30. Wang T, Fan Y, Koehler AV, Ma G, Li T, Hu M, et al. First survey of Cryptosporidium, Giardia and Enteroctozoon in diarrhoeic children from Wuhan, China. Infect Genet Evol. 2017;51:127–31.

31. Squire SA, Ryan U. Cryptosporidium and Giardia in Africa: current and future challenges. Parasit Vectors. 2017;10:195.

32. Abd El Kader NM, Blanco MA, Ali-Tammam M, Abd El Ghaffar Ael R, Osman A, El Shekhi N, et al. Detection of Cryptosporidium parvum and Cryptosporidium hominis in human patients in Cairo, Egypt. Parasitol Res. 2012;110:161–6.

33. Ayinmode AB, Fagbemi BO, Xiao L. Molecular characterization of Cryptosporidium in children in Oyo State, Nigeria: implications for infection sources. Parasitol Res. 2012;110:479–81.

34. Abu Samra N, Jori F, Caccio SM, Frean J, Poonsamy B, Thompson PN. Cryptosporidium genotypes in children and calves living at the wildlife or livestock interface of the Kruger National Park, South Africa. Onderstepoort J Vet Res. 2016;83:e1–7.

35. Eibach D, Krumkamp R, Al-Emran HM, Sarpong N, Hagen RM, Adu-Sarkodie Y, et al. Molecular characterization of Cryptosporidium spp. among children in rural Ghana. PLoS Negl Trop Dis. 2015;9:e0003551.

36. Al-Braiken FA, Amin A, Beeching NJ, Hohmell M, Hart CA. Detection of Cryptosporidium amongst diarrhoeic and asymptomatic children in Jeddah, Saudi Arabia. Ann Trop Med Parasitol. 2013;5:505–10.

37. Hijjawi N, Ng J, Yang R, Atoum MF, Ryan U. Identification of rare and novel Cryptosporidium GP60 subtypes in human isolates from Jordan. Exp Parasitol. 2010;125:161–4.

38. Alyousefi NA, Mahdy MA, Lim YA, Xiao L, Mahmud R. First molecular characterization of Cryptosporidium in Yemen. Parasitology. 2013;140:299–34.

39. Sulaiman IM, Hira PR, Zhou L, Al-Attai FM, Al-Shelahi FA, Shweiki HM, et al. Unique endemicity of cryptosporidiosis in children in Kuwait. J Clin Microbiol. 2005;43:2805–9.

40. Iqbal J, Khalid N, Hira PR. Cryptosporidiosis in Kuwaiti children: association of clinical characteristics with Cryptosporidium species and subtypes. J Med Microbiol. 2011;60:547–52.

41. Wielinga C, Thompson RC, Monis P, Ryan U. Identification of polymorphic genes for use in assemblage B genotyping assays through comparative genomics of multiple assemblage B Giardia duodenalis isolates. Mol Biochem Parasitol. 2015;201:1–4.