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Enteric Protozoa Associated with Acute Diarrhea in Hospitalized Children in Qatar

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Introduction

Diarrhea is the passage of three or more watery stool in a period of 24 hours (WHO, 2013). Types of diarrhea include acute watery diarrhea, acute bloody diarrhea known as dysentery and persistent diarrhea (WHO, 2013). It is caused by an infection of different pathogens including bacteria, viruses and parasites through fecal-oral transmission (WHO, 2013). Moreover, it can also be caused by food intolerance to certain food substances and as a side effect of certain medications such as laxatives (Burton & Ludwig, 2015). Diarrhea occurrence is most frequently associated with conditions of poor environmental sanitation and hygiene, poverty, inadequate water supply and limited education (Nelson & Masters, 2014). Worldwide, acute diarrhea disease is considered as the second cause of mortality and morbidity in children according to the World Health Organization (WHO, 2013). In 2012, WHO reported 1.9 million diarrheal cases in children aged under the age of five accounting for 18% of all deaths. The clinical manifestations of diarrhea in pediatric patients include abdominal pain, nausea, vomiting and fever (WGO, 2012 & Maas et al., 2014). Diarrhea in children can lead to many consequences such as malnutrition, diminished growth and impaired cognitive development (WGO, 2012). Severe diarrhea can also result in life-threatening dehydration (Galvao et al., 2013). Thus it is important to replace the fluid and electrolytes by oral rehydration solution. Diarrhea is usually self-limiting. However, in cases of diarrhea persisting for longer than 1 week, broad-spectrum antimicrobial agents are administered to treat bacterial and parasitic infection (Koletzko & Osterrieder, 2009). Intestinal protozoa that are most commonly associated with diarrhea in children include Blastocyst, *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium* species and *Entamoeba* species (Maas et al., 2014). Having updated information about the prevalence of these protozoan parasitic infections will aid in faster diagnosis and thus treatment. – Research question and objectives: Research Question: What are the most common protozoa and the risk factors for diarrhea in children under the age of 15 admitted to Hamad Medical Corporation (HMC).

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Objectives

To identify the prevalence of protozoa pathogen and the risk factors such as gender, age, season and geographical region associated with diarrhea in children.

Materials and methods

Study subjects and sample collection: A total of 391 Diarrheal stool samples were collected from March–July 2015 in a sterile container from pediatrics patients (0–15 years) admitted to HMC with diarrhea. The samples were transported on ice by Dr. Abu Madi's research group and frozen immediately at -70°C . All required ethical approvals for the project were obtained from Medical Research Centre. – **Stool examination:** To recover the DNA of the enteric pathogens samples were thawed at $+4^{\circ}\text{C}$ and 200 mg of the stool sample were weighed in a sterile 14 ml Falcon tube (BD Falcon). DNA was extracted using Qiagen miniamp stool kit (Qiagen, Germany) following manufacturer's instructions with minor modifications. The extracted DNA samples were analyzed by uniplex real-time PCR using Applied Biosystems Cyclor 7500. The protocol of the available literature has been used as a starting point. However, it was finalized by optimizing the concentrations of primer and probes and evaluating several cycles. The two different fluorescence reporters were used in which SYBR Green was used for Blastocyst, and TaqMan probe was used for *D. fragilis*, *G. lamblia*, *Cryptosporidium* and *Entamoeba*. For both reporters, Amplification reactions were performed in a 20 μL volume for each well with 17.5 master mix and 2.5 DNA template. However, the mastermix of SYBR Green consist of 10 μL SYBR Green Mastermix reagent (Qiagen, Germany), 2.2 μL of primer mix, and 5 μL of PCR grade water H₂O (Sigma, Germany). Whereas, Taqman reaction consists of 10 μL HotStar Taq Mastermix reagent (Qiagen, Germany), 1.3 μL of primer mix, 0.07 μL of probe and 6.2 of PCR grade water H₂O (Sigma, Germany). The initial incubation step is carried out at 95°C for 15 min to activate the HotStar Taq DNA polymerase, followed by a 40-cycle amplification program consisting of 15 s at 94°C , 30s at 57°C , 30s at 72°C , and a final extension step at 72°C for 30s. For each plate, internal positive controls were run consisted of positive samples brought from Hamad medical cooperation (HMC).

Definition of variables

All Birth dates and collection dates were recorded and the ages of the subjects were categorized into five classes by years, 1.1–1.9, 2.0–4.9, 5.0–9.9 and 10.0–14.9. The collection dates were classified according to the season into summer (May–October) and winter (November–April). The subjects in this study came from 34 different countries. For the purpose of analysis, the subjects were grouped into four geographical groups. These were as follows: Qatar (N= 97), from three countries in the Arabian Peninsula (N=16, Yemen, Saudi Arabia, Bahrain); from five countries in the Eastern Mediterranean (N=41, Jordan, Lebanon, Syria, Iraq, Iran); from 7 countries in Asia (N=131, India, Pakistan, Sri Lanka, Bangladesh, Nepal, Mauritania, Philippines); from 7 countries in Africa (N= 86, Nigeria, Egypt, Tunisia, Sudan, Djibouti, Eritrea, Moroccan); from 10 countries in Europe (N=20, Canada, Poland, UK, Greek, US, Holland, Spain, Italy, Venezuela, France) Statistical analysis: Prevalence data are shown with 95% confidence limits calculated using (<https://www.mccallum-layton.co.uk>). For determining the significance of different classes in each category, chi-square test was conducted using crosstabs descriptive statistics in IBM SPSS software. A p-value less than 0.05 is considered statistically significant.

Results

Screening for gastrointestinal pathogens using multiplex RT-PCR A total of 391 pediatrics patients participated in this study during the period of March–July 2015. Out of the 391 diarrheal patients (173 females and 218 males), 41 (10.7%) were positive for at least one protozoa (Table 1 and 2). Blastocyst was detected most frequently, in 4.1% (16/391), followed by *D. fragilis* in 3.3% (13/391), *Cryptosporidium* in 2.8% (11/391), *G. lamblia* in 2.0 (8/391) and *Entamoeba histolytica* in 0.3% (1/391) (Table 1). Most of diarrhea samples in the study came from the age group of 0–1 year (119/391) followed by 2–4.9 years (108/391), 1.1–1.9 years (105/391), 5–9.9 years (45/391) and 10–14.9 years (14/391) (Table 2). However, protozoa infections were highest at the age group of 5–9.9 years with a prevalence of 21.1% (Table 2 and Figure 1). *Blastocyst* and *Cryptosporidium* showed

the same pattern of infections among the age groups with the highest prevalence at the age group 5–9.9 years (Table 2). Whereas, *G. lamblia* and *D. fragilis* showed the highest prevalence among the age group of 10–14.9 (Table 2). Females had a higher prevalence than males in infections with Blastocyst (6.4%), *Cryptosporidium* (4.0%) and *G. lamblia* (2.9%) (Table 2 and Figure 2). Whereas, males had a higher prevalence than females in infections with *D. fragilis* (4.1) and *Entamoeba histolytica* (0.5%). A total of 34 countries categorized into 6 geographical regions were sampled in this study, but most of them were from Asia, Qatar & Africa regions (Table 2). However, the prevalence of protozoa infections was the highest among Europe (15%), followed by Qatar (14.1%), Arabian Peninsula (12.5%), Asia (9.9%), Africa (8.1%) and Eastern mediterranean (7.3%). Most of the diarrheal samples were collected during the summer season from May to July (Table 2 and Figure 3). However, protozoa infections had an overall higher prevalence during the winter season that is March and April (12.5%) (Table 2 and Figure 4).— Association of protozoa infections with age, gender, geographical distribution and season Although, most of the variables (i.e. gender, age, season) have shown a high value in one of the categories, the difference was not statistically significant ($p>0.05$) (Table 2). The only significant variables were the age in combined, Blastocyst and Cryptosporidium infections and the gender in Blastocyst ($p<0.05$) (Table 2). Blastocyst and Cryptosporidium infections affects the combined protozoa infections and have both the highest prevalence among the age group of 5–9.9 years with the prevalence of 15.6 and 11.1 respectively (Table 2). Blastocyst infections in Females have higher prevalence than male with a prevalence of 6.4% (Table 1).

Conclusion

This study has demonstrated that protozoa parasitic infections are still a public health problem in pediatrics patients with Blastocyst, *Dientamoeba fragilis* and *Cryptosporidium* being the most common respectively. Therefore, protozoa parasitic infections should be tested for in children complaining from diarrhea. The study also highlights the use of molecular techniques in diagnosis of protozoa parasitic infections.