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Significant Zoonotic Enteropathogens Associated with Childhood Diarrhea in Egypt

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Abstract: Diarrheal diseases remain one of the leading causes of childhood morbidity and mortality in most developing countries, with enteropathogenic *Escherichia coli* (EPEC) being one of the most important etiologic agents of infantile diarrhea in many of these countries. Eighty stool samples were collected from diarrheic children of less than 5 years of age attending the Teaching Pediatric Hospital (Abu Rish Hospital), Cairo, Egypt during the period from May 2015 to October 2015. Demographic characteristics, clinical symptoms and environmental data were recorded for each child using a questionnaire. Stool samples were analyzed for the detection of three significant zoonotic etiological agents of diarrhea including EPEC, Rotavirus (RV) and *Cryptosporidium* spp. and antibiotic susceptibility test of the EPEC isolates was carried out. Results showed that children were between one month and 55 months of age with a mean age of 11.6 + 0.9. Of these children, 42 (52.5%) were females and 38 (47.5%) were males with non-significant difference ($P \geq 0.5$). Twenty eight out of the collected 80 diarrhea stool samples (35%) were positive for isolation of investigated enteropathogens. The number of isolated pathogens was 34 including 12 EPEC (35.3%), 8 RV (23.5%) and 14 *Cryptosporidium* (41.2%). Six positive samples showed isolation of more than one pathogen. The serotypes of isolated EPEC were O111, O55 and O26. Antibiotic susceptibility test showed that EPEC isolates were highly resistant to cephalothin (91.7) and highly sensitive to ciprofloxacin (100%). It can be concluded that high personal hygiene by mothers and child minders in the preparation and storage of infants and children foods is an important factor to avoid food contamination by enteropathogens.

Key words: Children · Diarrhea · Enteropathogenic *E. coli* · Rotavirus · Cryptosporidium

INTRODUCTION

Gastroenteritis is one of the most important public health problems in both developed and developing countries. According to the World Health Organization [1], there are about two billion cases of diarrheal disease worldwide every year and 1.9 million children less than 5 years of age die from diarrhea each year, mostly in developing countries. This amounts to 18% of all the deaths of children under the age of five and 78% of all child deaths from diarrhea occur in the African and South-East Asian regions.

Three are three clinical pictures of diarrhea, each reflecting a different pathogenesis and requiring different approaches to treatment. These are acute watery diarrhea, dysentery and persistent diarrhea. Acute watery diarrhea lasts less than 14 days (most episodes last less than 7 days). Dysentery is defined as diarrhea with visible blood in feces. Persistent diarrhea begins acutely with unusually long duration (at least 14 days) [2].

Risk factors for diarrhea among children include age sex, geographic location, drinking from unprotected water supply, children's residence, maternal education and household economic status. While low birth weight, malnutrition and vitamin A deficiencies, poor sanitation, limited access to potable water, inappropriate breastfeeding practices contribute to the burden of disease, there continues to be the need to further document the socio-demographic correlates of diarrhea in order to inform policy and programmatic interventions that have potential to stem the prevalence of the condition [3].

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Human become infected directly or indirectly through contaminated food and fluids or via direct contact with the infected feces of human or animals. Because the majority of enteropathogens causing diarrhea are zoonotic, contact with animals in particular domesticated livestock (its primary host) constitutes great risk [2].

A wide range of bacteria, viruses and parasites can cause diarrhea. Bacteria like *Salmonella* spp. [4], *Campylobacter* spp., [5], *Shigella* spp., *Aeromonas, Vibrio cholera* and *E. coli* are significant causes of diarrhea [6].

The most common viruses related to diarrhea include rotavirus, adenoviruses, Norwalk virus, calicivirus, astroviruses and coronaviruses [7].

Intestinal parasites like *Giardia lamblia, Cryptosporidium* spp., *Entamoeba histolytica*, *Ascaris lumbricoides*, *Enterobius vermicularis* and *Hymenolepis nana* are one of the health problems. The incidence of these parasites is generally high in the tropical and subtropical countries, affecting mainly children of low socioeconomic groups [8].

Among the bacterial pathogens, enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC) are the most common causes of diarrhea in children in developing countries [9]. Dual infections with both pathogens have been noted. These strains are also an important cause of disease in nosocomial outbreaks, outpatient clinics, patients referred to hospitals and urban and rural areas [10].

This study was conducted to determine the prevalence of three significant zoonotic etiological agents of diarrhea (EPEC, Rotavirus and *Cryptosporidium*) in children attending the Teaching Pediatric Hospital of Faculty of Medicine, Cairo University and associated risk factors.

MATERIALS AND METHODS

This study was carried out in the Teaching Pediatric Hospital (Abu Rish Hospital), Cairo, Egypt during the period from May 2015 to October, 2015. Demographic information (age, sex and family income), clinical symptoms (fever, vomiting, dehydration) and epidemiological data (source of drinking water, contact with animals, mother washing hands) were recorded for each child using a questionnaire.

Sample Collection: Eighty stool samples from children aged < 5 years with clinical diagnosis of acute diarrhea collected from Oral Rehydration Treatment (ORT) ward and Outpatient department of Abu Rish Hospital (OPD) were included in the study. Stool samples were collected in sterile stool containers, transferred to Laboratory of the Zoonotic Diseases Department, National Research Centre, Egypt, on ice packs and processed within 4 h of collection.

Macroscopic examination of stool samples was done to investigate the presence of blood and physical characteristics such as color, appearance, odor and consistency.

Detection of Enteropathogenic *E. coli*: Collected stool samples were processed for EPEC according to the biochemical and serological techniques recommended [11]. All the EPEC strains isolated from diarrheal stool samples were confirmed by agglutination with specific antisera (*Escherichia coli* antisera Plasmatec laboratory product: UK) according to the instructions of the manufacturer.

Detection of Rotavirus: Fecal specimens were screened for the presence of RV using a rapid stool diagnostic test (Rota-Strip, Coris Bioconcept, Belgium) in accordance with the manufacturer's instructions.

Detection of *Cryptosporidium*: Direct smears prepared from fresh fecal samples were examined for the presence of *Cryptosporidium* using modified Ziehl Neelsen method [12]. Shedding of cryptosporidium oocysts may be intermittent, even in those patients with massive diarrhea, so that microscopically examination was performed on 3 stool samples to increase sensitivity [13].

Antimicrobial Susceptibility Testing of EPEC Isolates: Antimicrobial drug susceptibility testing of EPEC isolates was carried out using disc diffusion method [14] with the following 10 antibiotic discs: amoxicillin-clavulanic acid (30 µg), chloramphenicol (30 µg), ampicillin (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cephalothin (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), gentamicin (10 µg) and tetracycline (30 µg), (Oxoid).

Statistical Analysis: The results were statistically analyzed by calculating the mean and standard deviation and *P* value (significant at ≤0.05) for demographic, clinical and epidemiological data using SPSS 10 program for Windows (SPSS Inc., USA).

Table 1: Demographic, clinical and environmental characteristics of examined diarrheic children

Character		P value
Age (Months)	Mean: 11.6±0.9	
Sex		
Females	42(52.5%)	0.61
Males	38(47.5%)	
Maternal characteristics		
-Age:		
Age≤ 25y	46(57.5%)	0.05*
Age≥25y	34(42.5%)	
-Education:		
Illiterate	36(45%)	
Primary school	25(31.25%)	0.05*
Secondary school	11(13.75)	
College graduated	8(10%)	
-Feeding		
Only breast feeding	23(28.75%)	0.05*
Non breastfed children	57(71.25%)	
-Household income		
Low	65(86.25)	0.05*
Medium	15(18.75)	
Clinical characteristics		
-Temperature≥38°C (n, %)	59 (73.75%)	
-Vomiting		
Incidence (n, %)	64 (80%)	
Frequency (times/day)	2.6±0.4	
-Diarrhea		
Duration (days)	2-10	
Frequency (times/day)	3.9±0.3	
-Stool consistency		
Watery	63 (78.75%)	
Containing mucus	12(15%)	
Bloody	5(6.25%)	
- Dehydration	33(41.25%)	
- Antibiotic treatment (n, %)	29(36.25)	
Environmental factors		
-Drinking water		
Water tanks	9(11.25%)	
Tap.	71(88.75%)	0.05*
-Contact with animals	23(28.75%)	
-Mothers wash hands before feeding their children	57(71.25%)	

*Significant at (P=0.05)

Table 2: Number and percentage of investigated enteropathogens isolated from studied cases (no. 80)

Enteropathogen	No.	%
Enteropathogenic *E. coli* (EPEC)	12	35.3
Rotavirus (RV)	8	23.5
Cryptosporidium	14	41.2
Total	34	100

Table 3: Antibiotic susceptibility of isolated EPEC to different antimicrobial agents

Antibiotic	Resistance (no, %)
Amoxicillin-clavulanic acid (30 µg)	1(8.3)
Chloramphenicol (30 µg)	7(58.3)
Ampicillin (10 µg)	8(66.7)
Ceftriaxone (30 µg)	1(8.3)
Tetracycline (30 µg)	9(75)
Ciprofloxacin (5 µg)	-
Cefotaxime (30 µg)	2(16.7)
Cephalothin (30 µg)	11(91.7)
Cotrimoxazole (25 µg)	7(58.3)
Gentamicin (10 µg)	1(8.3)

RESULTS

Demographic, Clinical and Environmental Characteristics of Examined Diarrheic Children: The study included 80 children with acute gastroenteritis. The children were between one month and 55 months of age with a mean age of 11.6±0.9. Of these children, 42 (52.5%) were females and 38 (47.5%) were males with non-significant difference (P≥0.05). There were significant differences (P≤0.05) between breastfed and non-breastfed children, maternal age of ≤25 and ≥25 years, maternal education (illiterate, primary school, secondary school and college graduate), low and medium household income and drinking water from tap and water tanks (Table 1).

Prevalence of diarrheagenic enteropathogens: Results of the present study showed that 28 out of the collected 80 diarrhea stool samples (35%) were positive for isolation of investigated enteropathogens. The number of isolated pathogens was 34 including 12 EPEC (35.3%), 8 RV (23.5%) and 14 *Cryptosporidium* (41.2%) (Table 2). Six positive samples showed isolation of two or three pathogens. The serotypes of isolated EPEC were O111, O55 and O26.

Antibiotic susceptibility of EPEC isolates: Antibiotic susceptibility test showed that EPEC was highly resistant to cephalothin (91.7%), followed by tetracycline (75%), ampicillin (66.7%), chloramphenicol (58.3%) and cotrimoxazole (58.3%). Ciprofloxacin (100%), ceftriaxone (91.7%), amoxicillin-clavulanic acid (91.7%) and gentamicin (91.7%) showed high degree of sensitivity to the EPEC isolates (Table 3).

DISCUSSION

Diarrheal diseases remain one of the principal causes of childhood mortality and morbidity in low income

countries despite significant progress in our understanding of the pathogenesis of these diseases and in their management.

In the present study, 28.75% of the studied cases were breast fed compared to 71.25% were non breast fed (P≤0.5). Abdel Messih *et al*. [15] reported that breast feeding had a trend towards protection against diarrhea, as non- breastfed children are more exposed to infection through contaminated food and bottles. Diarrheagenic *E. coli* were more frequently isolated from samples from older infants than samples from younger infants, reflecting increased exposure to pathogens after 6 months of age, potentially resulting from the introduction of contaminated foods into the diet [16].

In the present study, the mean duration of diarrheal symptoms was 3.9 days±0.3 (range 2 - 10 days) and vomiting was noted in 80% of patients. On examination, 59 (73.75%) patients were febrile.

Youssef *et al*. [17] reported that vomiting, fever, abdominal pain and dehydration were the most common presenting symptoms in 19 studies in Egypt detecting *cryptosporidium* in individuals with diarrhea. Bonkoungou *et al*. [18] reported that fever (64%) and vomiting (61%) were the most common symptoms associated with diarrhea.

The epidemiologic data recorded that, tap water is the main source of drinking water (88.75%) and 71.25% of the mothers wash their hands before feeding their children. Also, it was found that 28.75% of the children have sheep in their house and play or have direct contact with pets.

Environmental information on children with diarrhea suggests three possible modes of transmission of infection: drinking contaminated water stored in overhead water tanks, person to person, or contact with infected animals [19].

In the present study 28 diarrhea stool samples (35%) were positive for isolation of investigated enteropathogens.

Higher results of isolation of enteropathogens from diarrhea in children were recorded by many investigators; Ngum *et al.* [20] (85.3%), Nitiema *et al*. [21] (57.9), El-Mohammady *et al*. [22] (48%), Bonkoungou *et al*. [18] (64%) and Laham *et al.* [23] (60.6%).

Lower result of the prevalence of diarrhea in children than the present study was obtained by Achi and Mbajiaka [24] who reported that 19.5% of samples from children with diarrhea were positive for pathogens isolation.

The difference in the prevalence might be due to the fact that our aim in the present study was isolation of three significant zoonotic enteropathogens (EPEC, RV and *Cryptosporidium*) as etiological agents of diarrhea in children less than 5 years of age.

Our results showed that five samples (6.25%) contained two or three enteropathogens. El-Mohammady *et al*. [22] and Laham *et al.* [23] found multiple pathogens in 9 and 11.4% of diarrhea cases, respectively. Mixed infections are an evolving problem in the epidemiology of diarrhea which makes it complicated to determine which pathogen is responsible for the disease, or whether there is an additive effect of each pathogen present in a coinfection [25].

Our results showed that the isolation rate of EPEC was 35.3%. Similar results with the present study was recorded by Sudershan *et al.* [26] who reported that isolation rate of EPEC from diarrheic stool specimens was 35%.

Lower results of isolation rate of EPEC were recorded by Nitiema *et al.* [21], 9.7 % and Nejma *et al.* [27], 13.7%.

In the concurrent work, the serotypes of the isolated EPEC were O111, O55 and O26. Similar result was recorded by Behiry *et al.* [28] who reported serotypes of the EPEC causing diarrhea in children less than five years in Cairo, Egypt to be of the same serotypes of our study.

EPEC is a major cause of diarrhea in developing countries. EPEC strains are diarrheagenic *E. coli*, which usually are classified by a combination of oligosaccharides (O), flagella (H) and capsular (K) antigens. Serotypes O55:H6 and O111:H2 were reported as most frequent isolates in different geographical areas [29]. They are associated with outbreaks of infantile diarrhea among children in developing countries. In contrast to the limited importance of EPEC in industrialized countries, studies in Brazil, Mexico and South Africa have shown that 30–40% of infant diarrhea can be accredited to EPEC [30].

Our results showed that the isolation rate of RV was 23.5%. Similar result was obtained by Sherchand *et al.* [31] (24.7%).

Rotavirus is the single most important etiological agent causing severe diarrhea in infants and young children under 5 years in both developing and developed countries [32]. Several reports estimated that group A rotaviruses cause about 140 million cases of acute gastroenteritis and one million deaths worldwide every year.

In the present work, 37.5% isolation rate of RV was reported by children with diarrhea in the age group 1-12months while the age group 13-24 months displayed a prevalence of 25%. Nejma *et al.* [27] observed that 41.0% of children 0-12months were most affected with rotavirus infection, while the age group 13-24 months had a prevalence of 30.7%.

Higher results of isolation rate of RV were reported by Ngum *et al.* [20] (32.8%), Nitiema *et al.* [21] (32.4%), Bonkoungou *et al.* [18] (30%) and Nejma *et al.* [27] (33.9%).

Lower results of RV prevalence in children with diarrhea were reported by many workers; El-Mohammady *et al*. [22] (14%), AlAyed *et al.* [33] (17.2%) and Laham *et al.* [23] (3.1%).

Viruses were found mainly in children of ≤ 2 years of age, whereas bacteria were equally prevalent among all the age groups [23].

Cryptosporidium is an obligate intracellular protozoa and *Cryptosporidium* infection is one of the important causes of diarrhea in children below 5 years of age. The main features of diarrhea are watery diarrhea of variable severity, abdominal pain and mild fever. In otherwise healthy individuals, cryptosporidium infection usually causes a self-limiting diarrheal disease [34].

Children appear to be susceptible to serious adverse consequences like stunting, lack of catch-up growth, cognitive and physical developmental delay even after asymptomatic infection with *C. parvum* [35].

Our results showed that the isolation rate of *Cryptosporidium* was 41.2% where it was higher in children older than 2 years of age (64.3%) than children less than 2 years of age (35.7%). Iqbal *et al.* [19] reported that Cryptosporidium oocysts were detected in 10% of children with diarrhea. Prevalence was highest (73%) in children >2 years of age compared with children <2 years of age.

Lower results of *Cryptosporidium* prevalence were reported by Carvalho-Costa *et al.* [36] (9.3%), El-Mohammady *et al*. [22] (5%) and Bera *et al.* [37] (27.4%).

The difference in the prevalence of studied pathogens may differ substantially between regions depending on local meteorological, geographic and socioeconomic conditions [38].

In developing countries, enteric bacteria and parasites are more prevalent than viruses and typically peak during the summer months. In addition to common enteropathogenic organisms, *Cryptosporidium* is indicated as a key causative agent of diarrhea in humans. Cryptosporidium infection usually causes a self-limiting diarrheal disease [34]. Although cryptosporidiosis may, in many cases, be terminated by self-limiting mechanisms, it could cause pathologies requiring preventive and therapeutic policies.

Results of the *in vitro* antibiotic susceptibility pattern of EPEC isolates showed that 11(91.7%), 9(75), 8(66.7), 7(58.3) and 7(58.3) isolates were resistant to cephalothin, tetracycline ampicillin, chloramphenicol and cotrimoxazole, respectively. On the other hand, EPEC exhibited high sensitivity to ciprofloxacin (100%), ceftriaxone (91.7%), amoxicillin-clavulanic acid (91.7%) and gentamicin (91.7 %).

Behiry *et al*. [28] reported that 57% of EPEC isolates from diarrheic children less than 5 years in Egypt were resistant to ampicillin and cotrimoxazole and (14.3%) to the third generation cephalosporins. Achi and Mbajiaka [24] recorded that isolated EPEC showed high resistance of 89.7 and 64.1% to Ampicillin and Ampiclox respectively. Ciprofloxacin and Ceftriaxone showed high degree of sensitivity to the EPEC isolates with rates of 92.1 and 82.1% respectively.

Fortunately, EPEC diarrhea is usually self-limited and rehydration is the most effective treatment. The use of antibiotics in general is of minor importance and has been criticized on the grounds of drug toxicity and the risk of increased of wide-spread antimicrobial resistance [39].

CONCLUSIONS

In this study *Cryptosporidium* (41.2%) showed the highest prevalence among the studied diarrheic children followed by EPEC (35.3%) and then RV (23.5%).

Application of teaching health programs for maternal knowledge about breast feeding and suitable time for weaning is important for the prevention and control of diarrhea. High personal hygiene by mothers and child minders in the preparation and storage of infants and children foods is an important factor to avoid food contamination by enteropathogens.

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