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Research Article

Microorganisms and viruses causing diarrhea in infants and primary school children and their relation with age and sex in Zakho city, Kurdistan Region, Iraq

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ABSTRACT

Background: Diarrheal diseases in children are a major public health concern in developing countries. Diarrheal infection spreads through contaminated food or drinking-water, or from person-to-person as a result of poor hygiene. This study was conducted to investigate the incidence of different microorganisms associated with diarrhea in infants and primary school children and their relation to sex and age in Zakho city, Kurdistan region, Iraq.
Methods: During the period from September 2013 to May 2014, 600 diarrheic samples were collected from both sexes and different ages (<2 to 12) years; these samples were subjected to various diagnostic tests in the Microbiology Laboratories/Biology Department/Faculty of Science/University of Zakho.
Results: Out of 600/479 were positive for one or more types of microorganisms including 265 (55.32%) males and 214 (44.67%) females. Among the positive 479 samples, the highest rate of prevalence was with both bacteria and parasites (57.33and 57.00%, respectively), followed by viruses (10.33%) and only1.16% with fungi. The most prevalent enteric microorganisms were found to be *E. coli* (62.5%), followed by *E. histolytica* (46.19%), *G. lamblia* (42.10%) and the lowest *H. nana* (0.87%). The mixed prevalence were documented in 179 (37.36) cases, with the most common correlation between bacteria and parasites in 76 (42.45 %) cases. All ages showed high rates of prevalence with both bacteria and parasites, the highest bacterial cases being among ages >4-8 years (65.38 and 64.07%), while parasites were among >6-10 years (64.67 and 60.11%). High viral prevalence were recorded among ages <2 to 6 years with the peak among <2 years (40.54%).
Conclusions: From this study we conclude that about 57% of diarrheal cases were associated with bacteria, parasites, and viruses with *E. coli, E. histolytica, G. lamblia* and rotavirus as leading microorganisms. The mixed prevalence with two or more microorganisms was documented in 179 (37.36%) out of 479 positive samples and the rate of microbial prevalence was found to be gender and age dependent.

Keywords: Microbial diarrhea, Bacteria, Parasites, Viruses, Infants and children, Sex, Age

INTRODUCTION

Diarrheal diseases in children are a major public health concern in developing countries. Diarrhea has been estimated to cause 1.5 million deaths, of which 21% in children under the age of 5 and 15% in children above the age of 5 years worldwide.[1,2] Diarrheal infection spreads through contaminated food or drinking-water, or from person-to-person as a result of poor hygiene. There are three clinical types of diarrhea: Acute watery diarrhea lasts several hours or days, it includes cholera; bloody diarrhea also called dysentery, is the diarrhea that lasts 14

days or longer and chronic diarrhea which is a common condition lasting more than 4 weeks and occurs in up to 3-5% of the population.[3,4]

Diarrheal diseases due to unsafe water and lack of sanitation are the biggest causes of morbidity and mortality in under five year's children in the world especially in poor countries.[5,6] A child dies every 15 seconds from diarrhea caused largely by poor sanitation and contaminated water supply.[7]

Diarrhea among children can be caused by many infectious agents like bacteria, parasites, viruses and fungi, in addition to other non-infectious agents like malnutrition and malignancies.[8]

Very limited information are available on diarrheal cases in children in Zakho city, Kurdistan Region of Iraq, therefore, the aim of this study was to perform a survey and to identify the causative agents of diarrhea in infants and children of both sexes and different ages.

METHODS

Sample collection

In this study 600 stool samples were collected during the period from September 2013 to May 2014, from infants and children of both sexes and from different ages ranged from< 2 to 12 years, who attended the outpatient clinic in Zakho general hospital, and from children of primary schools in different regions of Zakho city. After collection, all samples were kept in clean labeled screw cup containers containing 5 ml of normal saline, except for *Cryptosporidium* oocysts and *Enterobius vermicularis* which were collected and processed differently. Then all samples were kept in ice box and transferred to microbiology laboratory within 2 hours of collection to be processed for examination and identification. All stool samples were labeled and full information has been taken from each patient.

Sample processing

In the laboratory, the collected samples were processed following the standard laboratory protocol[9] in the Public Health Research laboratories, as:

Macroscopical examination: Stool samples were examined visually and the followings were recorded on a special form in addition to personal information required for the study, the color, consistency, presence of blood and mucus and any other abnormalities were observed macroscopically and documented.

Microscopical examination: This was performed by 2 methods:

1. *Direct wet mount method*

A small fleck of the specimen was placed in a drop of normal saline on one side of a clean slide and a drop of Lugal's iodine on another slide side, they were mixed thoroughly by a wooden stick, then they were covered with a cover slip and examined by light microscope, firstly with 10x, 40x then 100x to look for parasites, pus cells, leukocytes, RBCs, epithelial cells and others. From each sample 3 slides were taken from different parts of the sample and examined.

2. *Concentration technique (Zinc sulphate floatation)*[10]

This method was used to detect protozoan cysts, helminthes ova and larva, in which about 2 gm from each stool sample was mixed with 10-12 ml of normal saline. The mixture was strained through two layers of wet surgical gauze, and centrifuged for two minutes at 1500-2000 rpm. The supernatant fluid was decanted, the sediment was resuspended in normal saline and centrifuged again. This process was repeated for three times. A centrifuge tube was filled with zinc sulfate close to the rim and covered with a cover slide and centrifuged again at 2500 rpm for one minute. The cover slide was transferred to a slide containing one drop of lugal's iodine, then examined under 10, 40 and 100x. The detected organisms were recorded.

3. *Acid fast stains (Ziehl-Neelsen)*[11]

This method was used to detect oocysts of *Cryptosporidium* spp. For each individual, one fresh fecal specimen from early morning discharge was collected and prepared for examination. Each specimen was collected in 10% buffered formalin in a clean wide-mouthed plastic container and was subjected to concentration technique, stained with modified Ziehl-Neelsen and examined under the microscopic for the identification of *Cryptosporidium* oocysts.

4. *Scotch tape method*[12,13]

This method was used for diagnosis of *E. vermicularis* "scotch tape" test, which was used for children aged 2-6 years and performed by sticking a clear cellophane tape onto a wooden stick, and then doubling it over so that the sticky side points outwards. The stick with the tape was pressed against the perianal skin, allowing eggs to adhere to the tape. Then the swap was placed in a test tube containing about 2ml of normal saline then transferred to the laboratory for processing and the results were reported.

Bacteriological isolation (cultivation)

After delivering the stool samples to the laboratory, the bacteriological examination and characterizations were performed as following.

Each stool sample was cultured on the following media: Neutrient, MacConcky, Blood, and *Salmonella shigella* agar (SSA). All these media were prepared according to manufacturer instructions. To each of these media, a small amount of the stool from each sample was streaked, and then incubated at 37°C for 18-24 hours. Isolated colonies of positive cultures were sub-cultured on selective media (Methyl Red and Voges-Proskauer, Simmon citrate, and peptone water) then diagnosed.

Identification of viruses[14]

Cer Test Rota-Adenovirus kits were used.

1. The end of the cap was cut and about 1gm of stool sample was placed in the stool collection tube containing the known antibody and shacked thoroughly in order to assure good sample dispersion.
2. The Cer Test Rota-Adenovirus card device was removed from its seal bag just before using it.
3. A separate stool collection tube and device were used for each sample or control. Four drops or 100 µl of the stool mixture was dispensed into the circular window marked with an arrow.
4. The results were read at 10 minutes by observing the coloring bands:

Negative results showed only one green band which appeared across the central window in the site marked with the letter C (control line).

If it is Rota virus positive, a red band (Rotavirus test line) also appears in the site marked with the letter T in addition to the green control band.

Adenovirus positive show a blue band (Adenovirus test line) which appears in the site marked with the letter T in addition to the green control band. If rotavirus-adenovirus positive, all the lines appears (a green control band in the control region, a red band and a blue band in the result regions).

Isolation and identification of fungi

After gram staining, the yeast isolates were subcultured on candida chrome agar for the identification of *Candida* species, and incubated at 35°C for 24-48 hours, to permit the development of colored colonies. The presumptive identification was made by color and morphology of the colonies; the isolates were further identified microscopically and morphologically.

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism 5 program. The chi-square test was used and $P<0.05$ was considered to be statistically significant.[15]

RESULTS

Distribution of microorganisms in diarrheic samples

The distribution of enteric microorganism in the examined diarrheal samples is shown in Table 1. As it is clear from the table, 479/600 examined stool samples were positive for various types of microorganism and some of them have more than one type of microorganisms. The highest rate of prevalence was with both bacteria and parasites (57.33and 57.00%, respectively), followed by viruses (10.33%) and only 1.16% of samples were with contained fungi. Regarding the identified microorganisms, among bacteria, the highest prevalence was with *E. coli* (62.5%), for parasites high prevalence was reported with both *E. histolytica* and *G. lamblia* (46.19 and 42.10%, respectively), and rotavirus (69.35%) (Table 2).

As indicated in Table 1, diarrhea is caused by a wide variety of enteric microorganisms including bacteria, parasites, viruses, and fungi, but in the present study it appeared that the main causative agents were both bacteria and parasites.

The prevalence of mixed microorganisms

Table 3 shows the frequency of mixed microorganisms encountered in the examined diarrheal samples. The mixed prevalence with two or more microorganisms was documented in 179 (37.36%) out of 479 positive samples. The most common correlation of two microorganisms was between bacteria and parasites which was found in 76 (42.45 %) cases, followed by bacteria and bacteria (two different species) in 25 (13.96%) cases, bacteria and viruses 24 (13.40%) cases, parasites and viruses in 23 (12.84%) cases, parasite-parasite (two different species) in 7 (3.91%) cases, and bacteria-fungi in 3 (1.67%) cases only. However triple prevalence with bacteria, bacteria and parasite and bacteria, parasite and virus was reported in 6 (3.35%), cases for each of them, bacteria, parasite and fungi in 2 (1.11%) cases, and parasite, parasite and virus in one (0.55%) case. The most encountered microorganisms in mixed cases were *E. histolytica*, *G. lamblia*, *E. coli and Klebsiella spp*.

The distribution of microorganisms according to age

The distribution of microorganisms among different age groups is shown in Table 4. It is obvious from the table that the rates of prevalence with bacteria and parasites were high among all the groups (from <2-12 years) with the highest (65.38 and 64.07%) at the ages above 4-8 years, respectively. Regarding diarrhea caused by viruses, the rate was high among the age groups from less than 2 - 6 years with the peak in less than 2 years. The differences in rates of prevalence with bacteria, parasites and viruses among the age groups was statistically extremely significant ($p<0.0001$).

Relationship between gender and age for diarrhea

The relationship between the gender and age among different age groups is shown in Table 5. Among the positive cases, it is obvious from the results that more males were positive as compared to females, since 265 (55.32%) males versus 214 (44.67%) females/out of 479 positive cases over all age groups. The highest prevalence rate is at the ages from less than 2 years to 8 years, while at ages above 8 to 12 years the rate is decreased, but still it is high since, at the age of 12 years 60.76% of the examined children were positive (Table 5). All these differences were statistically significant ($p<0.05$) between age and gender.

Table 1: The distribution of various microorganisms in the examined diarrheic stool samples (No.=600).

Types of microorganisms							
Bacteria		**Parasites**		**Viruses**		**Fungi**	
No. positive	**%**	**No. positive**	**%**	**No. positive**	**%**	**No. positive**	**%**
344	57.33	342	57.00	62	10.33	7	1.16

Table 2: Types of microorganisms recorded in diarrheic samples (No.=479).

Microorganisms	Number positive	%
Bacteria		
E. coli	215	62.5
Klebsiella spp.	117	34.01
Pseudomonas spp.	7	2.03
Shigella spp.	5	1.45
Total	344	90.77
Parasites		
E. histolytica	158	46.19
G. lamblia	144	42.10
Cryptosporidium oocyst	28	8.18
E. vermicularis	9	2.63
H. nana	3	0.87
Total	342	90.24
Viruses		
Rotavirus	43	69.35
Adenovirus	19	30.64
Total	62	16.36
Fungi		
Candida albicans	7	1.16

Table 3: The frequency of mixed microorganisms among 479 cases of diarrhea.

	Frequency	%
Bacteria+Parasite		
E.coli+E.histolytica	37	20.67
Klebsiella spp.*+E. histolytica*	19	10.61
Shiglla spp.*+E. histolytica*	5	2.79
E.coli+G. lamblia	15	8.37
Total	76	42.45
Bacteria+Bactria+Parasite		
E.coli+Klibsiella spp.*+E. histolytica*	6	3.35
Bacteria+Virus		
E. coli+Rotavirus	13	7.26
E. coli+Adenovirus	4	2.23
Klebsiella spp.+Rotavirus	7	3.91
Total	24	13.40
Bacteria+Bactria		
E. coli+Klebsiella spp.	22	12.29
E.coli+Pseudomonas spp.	3	1.67
Total	25	13.96
Parasite+Virus		
E. histolytica +Rotavirus	17	9.49
E. histolytica +Adenovirus	2	1.11
G. lamblia+Rotavirus	4	2.23
Total	23	12.84
Parasite+Parasite		
E. histolytica+G. lamblia	2	1.11
E. histolytica+H. nana	1	0.55
G. lamblia+Cryptosporidium oocyst	2	1.11
G. lamblia+E. vermicularis	2	1.11
Total	7	3.91
Bacteria+Parasite+Virus		
E.coli+G. lamblia+Rotavirus	6	3.35
Bacteria+Bactria+Parasite		
E. coli+Klebsiella spp.*+G. lamblia*	6	3.35
Bacteria+Fungus		
E. coli+Candidaalbicans	3	1.67
Bacteria+Parasite+Fungus		
E. coli+E. histolytica +C. albicans	2	1.11
Parasite+Parasite+Virus		
G. lamblia+Cryptosporidium oocyst*+Rotavirus*	1	0.55
Total number of cases	179	37.36

Table 4: The distribution of enteric microorganisms among different age groups (No.=600).

Age groups (years)	No. examined	Types of microorganisms							
		Bacteria +ve		Parasites +ve		Viruses +ve		Fungus +ve	
		No.	%	No.	%	No.	%	No.	%
< 2	37	22	59.46	21	56.76	15	40.54	2	5.41
>2-4	34	20	58.82	18	52.94	10	29.41	1	2.94
>4-6	26	17	65.38	12	46.15	8	30.77	1	3.85
>6-8	167	107	64.07	108	64.67	9	5.39	1	0.60
>8-10	178	105	58.99	107	60.11	10	5.62	1	0.56
>10-12	158	73	46.20	76	48.10	10	6.33	1	0.63
Total	600	344	57.33	342	57.00	62	10.33	7	1.17

*P<0.0001

Table 5: Relationship between gender and age groups for diarrhea.

Age groups (years)	No. examined	Inf. total		Gender			
				Inf. male		Inf. female	
		No.	%	No.	%	No.	%
<2	37	36	97.30	20	54.05	16	43.24
>2-4	34	33	97.06	19	55.88	14	41.18
>4-6	26	25	96.15	13	50.00	12	46.15
>6-8	167	150	89.82	82	49.10	68	40.72
>8-10	178	139	78.09	79	44.38	60	33.71
>10-12	158	96	60.76	52	32.91	44	27.85
Total	600	479	79.83	265	55.32	214	44.67

*P<0.05

DISCUSSION

The source of drinking water is very important for human health, high rates of infection may be due to the drinking of tape water (personal observations as noted during this study), the lack of clean water, and, improper fecal disposal leads to contamination of groundwater especially in the absence of water filtration or purification processes, and this can help the widespread of some bacteria and parasites among children.[30,31] High rate (79.83%) of examined stool samples were positive for various types of microorganism some of them have more than one type of microorganisms, the highest rate of prevalence was with both bacteria and parasites (57.33and 57.00%, respectively), followed by viruses (10.33%) and only 1.16% of the samples contained fungi. In a similar study performed in Baghdad, a comparable rate of bacterial prevalence (58.33%) was reported among children,[16] while in a study in Duhok, higher rate of bacterial prevalence which was 81.61 % with high rates of *E. coli* and *Kleibsiella* spp. and lower rates of parasitic and fungal prevalence (27.97 and 0.95% respectively). Regarding parasites, also *E. histolytica* and *G. lamblia* predominate in these cases.[17] On the other hand, a higher bacterial prevalence (89%) in Kirkuk city has been reported.[18] However, a lower prevalence rate (34.5%) of bacteria was recorded among infants and children in Erbil.[19]

The rate of parasites in this study was comparable to that found among children in Baghdad which was 57.8%, also with high prevalence of *E. histolytica* and *G. lamblia*.[20] On the other hand, lower rates of parasite prevalence among infants and children were reported in various parts of Iraq, such as Erbil, Wasit and Kerbala, which were 30, 34.6 and 38.5%, respectively.[21-23] While, in Iran a higher rate (78.6%) with parasitic prevalence among children has been recorded.[24] The rate of viruses in this study was comparable to that found among children in Saudi Arabia which was 10.88%,[25] while, in Babylon and in Duhok higher rates (50.5 and 13.21%, respectively) of viral prevalence among children have been reported.[17,26] Furthermore, in Tehran (Iran) a lower rate (8.97%) of viral prevalence among children has been reported.[27] Regarding fungi, a very low rate (1.16) has been reported in the present study, similarly in Duhok province also a low rate (0.95%) of fungal prevalence among infant and children has been recorded.[17] On the other hand, very high rates with fungal prevalence have been reported in some studies in different parts of Iraq, such as Kirkuk, Tikrit, and Baghdad. which were 25, 2.5, and 29.15%, respectively.[18,28,29]

The mixed prevalence with two or more microorganisms was documented in 179 (37.36%) out of 479 positive samples. The most common correlation of two microorganisms was between bacteria and parasites which was found in 76 (42.45 %) cases. Comparable rate of mixed prevalence which were mostly with bacteria and parasites have been reported among children in Kirkuk (38.34%),[18] while much lower rates (21.3, 10.57 and 14.4%) with mixed microorganisms among children in Sweden, Kirkuk and Turkey, respectively were recorded[32-34] in Duhok also low rate of mixed microorganisms (25.54%) but with higher frequency of bacteria and parasites as observed in the present study has been reported.[17]

High rates of microbial prevalence have been reported among the ages >6-10 years. Similarly high rates of prevalence among these ages have been reported in Erbil, which was 11.20%.[35] Also in some other studies such as in Thi-Qar, a high rate (33.6%) of parasitic prevalence in this age group (6-10 years), has been reported,[30] but in AL-Mahmoudyia area/Baghdad province even a higher rate (42%) in children aged 8-10 years has been reported.[19]

The high rates of microbial prevalence among children at this age may be due to the fact that they were more involved with outdoor activities, therefore, they were subjected to higher risks of exposure in addition to low sanitary service, low education of mothers, improper water supply, absence of regular hygienic toilets, malnutrition and other socioeconomic factors, all can affect the children health.[21,36]

High prevalence rates (59.46, 56.76 and 40.54%) with bacteria, parasites and viruses among the age group less than 2 years from the number of examined samples at this age, may be due to exogenous factors such as reduction of breastfeeding alone with the increase in food supplementation in the second year of the life as supplementary food can be contaminated during preparation under poor hygienic conditions.[37]

Over all ages, males showed higher rates of microbial prevalence, with the highest being among ages from <2-8 years. Similarly in Baghdad and Duhok high rates of prevalence with different microorganisms in this age group have been reported.[17,29]

Data from other parts of Iraq such as Baghdad, Kirkuk and Duhok showed similar observations in which higher rates of diarrhea occurred in males than females.[17,29,33]

High rates of diarrhea caused by parasites among these ages has been reported by many authors from Baghdad and Duhok with the highest being in males.[18,38,39] Generally male children are more active and more frequently play outside their homes, whereas, female children are less likely to play outside and are more likely to eat home cooked food in addition to mothers level of education and employment status, father's level of education, mother's nutritional knowledge, all these factors are involved in the higher prevalence rate of microorganisms at this age.[40] The slight decrease in the prevalence rate in older children could be attributed to the fact that most enteric microorganisms stimulate at least partial immunity against reported cases.[41]

CONCLUSION

From this study it has been concluded that about 57% of diarrheal cases were associated with bacteria, parasites, and viruses with *E. coli*, *E. histolytica*, *G. lamblia* and rotavirus as leading microorganisms, The mixed prevalence with two or more microorganisms was documented in 179 (37.36%) out of 479 positive samples and the rate of microbial prevalence was found to be gender and age dependent.

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