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Pathogenic Bacteria and Parasites Associated with Diarrhea in Infants and Children in Zakho City, Kurdistan Region, Iraq

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ABSTRACT

Intestinal parasites and bacteria especially enteropathogenic Escherichia coli (EPEC) are important causative agents responsible for persistent diarrhea in infants and children worldwide due to their high occurrence in both the hospitals and community settings. However, sporadic outbreaks by these microorganisms occur globally. This study was conducted from August 2021 to January 2022. In this study 500 diarrheic stool samples were collected from both genders and various ages (6 month to 12 years) from outpatient in Zakho hospital to identify the causative agents of diarrhea in infants and children of both gender and different ages. In addition to find out the relationship of pathogenic bacteria and parasites with gender and age

In this study the total rate of infection was 64.2% (321/500), considerable number of samples were positive for more than one species of microorganisms, elevating the rate to 89%, with the highest rate (62%) being with bacteria among which E. coli was the dominant species (74.84%), while the rate with parasites was 27%, with E. histolytica being the dominant species (45.93%). Other recorded bacterial species with their rates are: Klebsiella spp. (15.16%) Pseudomonas spp. and Shigella spp. at rates of 6.13% and 3.87%, respectively. While other parasites included: Cryptosporidium spp. (34.07%), Blastocysts spp. (13.33%), G. lamblia (5.93%) and H. nana (0.74%). Single infections were reported in 61.68% (198/321) of the positive cases with the highest rate (49.53%) of bacteria and only 12.15% with parasites. The dominant organisms in single infections were E. coli and the oocysts of Cryptosporidium spp. at rates of 81.13% and 61.54%, respectively. The mixed infections were documented in 38.32% (123/321). Among these, 67.48% (83/123) showed a combination between bacteria and parasites. The most frequent microorganisms encountered in mixed infections were E. coli and E. histolytica at rate of 33.33%. The total rate of infection in males was higher than that in females (59.19 vs 40.81%), with the highest (77.89%) being among the age group 6 months-2 years. In each gender separately, males of the age group 6 months to 2 years showed the highest rate (62.16%,) whereas females of the age group 9-12 years had the highest rate (51.16%). Statistically the relationships between the rate of infection, age and gender were non-significant (P = 0.294). In conclusions it's obvious that 89 % of diarrheal cases were associated with pathogenic bacteria, and parasites, with E. coli, Klebsiella spp., E. histolytica, and Cryptosporidium spp. oocysts as leading microorganisms. Most infections occurred with one type of microorganisms, and the prevalence of microbes was found to be gender and age independent. Therefore, it is recommended to disseminate the health education and sanitary application programs among the community.

KEY WORDS: Microbial diarrhea, Bacteria, Parasites, Infants and Children, Gender, Age.

1. Introduction

Diarrheal disease is a global problem especially among children under five years of age and is considered as the fourth-leading cause of death in developing countries since its responsible for 499,000 deaths representing 8.6% of all deaths in this age group (GBD, 2015 and Risk Factors Collaborators, 2016). Diarrhea can lead to serious, lifelong health consequences such as environmental enteric dysfunction (EED), growth faltering, impaired cognitive development, and reduced immune response to infection and vaccinations (Guerrant *et al.*, 2013). The causative pathogenic agents of diarrhea may vary depending on the geographic location, duration of contact, or the population sampled (Lindsay *et al.*, 2015). These infections are believed to be different in the developing countries compared to the developed one regarding to several features, including earlier age of onset, multiple repeated exposures, greater diversity of pathogens, nutritional status of the host, and other factors, such as co-infection, diet, and genetics (Heidt *et al.*, 2014; GBD 2015 and Eastern

Mediterranean Region Diarrhea Collaborators, 2018).

The etiological agents of diarrhea include a wide range of microorganisms such as viruses, bacteria, and parasites (Bueris *et al*., 2007). They are transmitted by ingesting contaminated food, drinking contaminated water, or direct person-to person contact, and from contaminated hands (feco-oral-route). Human hands usually harbor microorganisms both as a part of person's normal microbial flora as well as transient microbes acquired from the environment especially in rural areas (Navaneethan and Giannella, 2008 Tambekar *et al*., 2009; Kotloff *et al*., 2013; Siddiqui *et al*., 2020 and Nguyen *et al*., 2021). Among these pathogens, diarrheagenic *E. coli* play a major role in causing diarrhea in children less than 5 years (Thakur *et al*., 2018; Ramakrishnan *et al*., 2018 and Saka *et al*., 2019). In usual cases, diarrhea normally lasts less than 7 days, but if it last more than 14 days, it is referred to as protracted diarrhea (Sumathi *et al*., 2018).

Watery diarrhea, stomach cramps, nausea, or vomiting, and occasionally fever are all symptoms of viral gastroenteritis (Demers-Mathieu *et al*., 2018). Therefore, the type of diarrhea can be severe in some cases due to confections by more than one type of microorganisms (Owada, 2019). When the microbial agent is bacteria, *E. coli* is considered as the major cause, especially in infantile diarrhea (Spano *et al*., 2017 and Ramakrishnan *et al*., 2019).

There is a dearth of information on diarrheal causes in infants and children in Zakho city, Kurdistan Region of Iraq. Therefore, the objectives of this study were to identify the causative agents of diarrhea in infants and children of both gender and different ages. With special emphases bacteria to the relationship between and parasites and to correlate the obtained data with gender and age.

2. Materials and Methods

2.1 Sample Collection

In the current study, 500 fresh stool specimens were collected during the period from August 2021 until January 2022, from infants and children of both genders and from various ages ranged from 6 month to 12 years, who visited the Zakho General Hospital's. Before collecting samples, a verbal consent was taken from the infant or the child mother in addition to an approval from the ethical committee of the general directorate of Health in Zakho city (Accession No. 698/3 and 2021/08/09). Each patient was requested to place about 5g of stool in a sterile screw cupped container fully labeled with the name, gender; age and type of stool according a questionnaire designed for the study. At each visit the collected specimens were kept in cool box and transferred within one to two hours to microbiology laboratory for examination and identification.

2.2 Sample Processing

In the laboratory, the collected samples were processed following the standard laboratory protocol (WHO, 2007) in the Public Health Research laboratories, as following:

2.2.1 Macroscopical Examination

The macroscopic examination of fresh stool specimen was performed to observe the macroscopic appearance of the stool such as, its consistency (formed, soft, watery, semiliquid and mucoid), color (brown, yellow, green, black, and red), blood (without, few, moderate, present, and abundant), mucus (not seen, few, moderate, present, and abundant) and the presence of parasitic stages such as proglottids, larvae or adult worms (Garcia, 2007).

Microscopical examination: This was performed by 3 methods:

2.2.1.1 Direct saline wet-mount preparation

A little amount from each stool sample was placed on the center of a clean microscope slide, if the specimen

was solid one to two drops of normal saline were added, mixed and smeared. Usually, two smears were prepared on the same slide for each specimen, one without stain, and to the second one to two drops of Lugol's iodine were added. If examined with oil immersion lens, the coverslip was sealed by adding to its four corners a warm mixture of paraffin and petroleum jelly (in a ratio of 1:1) as indicated by CDC (2016). Then, the smears were examined with the low and high-power objectives (10x, 40x or 100x if required) (Zeibig, 2014). From each stool specimen at least three slides from various parts of the specimen were made for the detection of parasitic stages.

2.2.1.2 Concentration technique (Zinc sulphate floatation):

In order to increase the probability of detection of protozoan cysts, helminths eggs, and larvae using this procedure, about 2 g from each stool specimen was mixed with 10–12 ml of normal saline, and then the mixture was strained through two layers of wet surgical gauze in to a centrifuge tube. The mixture was 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. Zinc sulfate solution (S.G. of 1.18) was poured into the centrifuge tube, filled up to the rim, covered with a cover slide, and centrifuged again at 2500 rpm for one minute. The cover slide was moved and placed on a slide with one drop of Lugol's iodine, inspected at 10 x, 40 x, or 100 x oil immersion lens. For viewing the slide under 100 x, the coverslip was sealed by adding to its four corners a warm mixture of paraffin and petroleum jelly (in a ratio of 1:1) as indicated by CDC (2016).The detected organisms were recorded (Faust *et al*., 1938).

2.2.2.3 Modified Ziehl-Neelsen (MZN) preparation for detection of *Cryptosporidium* spp. oocysts

From each stool specimen, a smear was prepared directly. Then, allowed to dry in air or on the slide warmer at 60 °C until dried. Then fixed with absolute methanol for 30 seconds, then the slide was flooded completely with kinyoun carbol fuchsine for one minute, rinsed with distilled water, detained with acid alcohol for 2 minutes, rinsed with distilled water. Counterstained with Malachite green for 2 minutes, rinsed briefly with distilled water, dried on slide drier for about 5 minutes. Mounted with DPX, or Canada balsam, covered by a coverslip and examined using 40 x objectives. To confirm internal morphology, 100 x oil immersion objective was used (Markey *et al*., 2013 and CDC, 2016).

2.2.2 Bacteriological isolation (cultivation)

After delivery the fecal specimens to the laboratory, the following bacteriological examinations were performed. A loopfull from each stool specimen was streaked on each of the following media: MacConkey agar, Sorbitol-MacConkey agar, and Eosin methylene blue agar (EMB), Salmonella and Shigella agar (SS agar) and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS). All medium were prepared according to the manufacturer instructions and then incubated at 37°C for 18 to 24 hours. Then the isolated pure colonies were further subcultured on different media for studying colony morphology, stained by Gram's stain for microscopical features and lastly subjected to different biochemical tests such as indole, methyl red, Voges-Proskauer, citrate utilization, TSI, oxidase and motility (Alexander and Strete, 2001 and Leboffe and Pierce, 2011) for their identification.

2.2.3 Data Analysis

The obtained epidemiological data were analyzed using the SPSS statistical analysis program (version 25), and the Chi-square (X^2) test was used to assess the probability value, $P< 0.05$, considered significant.

Table 1: different biochemical test on the different bacteria isolation in these studies

Isolated bacterial species	Oxidase	Indole	M.R	V.P	Simmon Citrate	TSI	Motility
E. coli	-	+	+	-	-	A/Agas	Motile
Klebsiella spp.	-	-	-	+	+	A/Agas	Non-motile
Pseudomonas spp.	+	-	-	-	+	Alk/ Alk	Motile
Shigella spp.	-	+	+	-	-	Alk/A	Non-motile

2.2.4 Distribution of bacteria and parasites in diarrheic stool specimens

The distribution of enteric bacteria and parasites in the examined stool specimens is shown in Table (2), as it is demonstrated in the table, 64.2% (321/500) of the identified stool specimens were positive for several types of enteric bacteria and parasites. Some of the specimens were infected with more than one species of these microorganisms.

Table (2): The distribution of bacteria and parasites in the examined diarrheic stool specimens.

No. of Examined Specimens	No. infected	Total %	Types of microorganisms			
			Bacteria		Parasites	
			No. infected	%	No. infected	%
500	321	64.2	310	62	135	27

Table (3). Showing the species of bacteria and parasites recorded and their percentages among examined stool specimens (n=500).

Type of recorded microorganism		No. of infected	%
Bacteria	*E. coli*	232	74.84
	Klebsiella spp.	47	15.16
	Pseudomonas spp.	19	6.13
	Shigella spp.	12	3.87
Sub-total		310	62
Parasites	*E. histolytica*	62	45.93
	Cryptosporidium	46	34.07
	Blastocysts	18	13.33
	G. lamblia	8	5.93
	H. nana	1	0.74
Sub-total		135	27
Total number of recorded microorganisms Including double infections		445	89

Table (3) shows the species of recorded microorganisms, here the rate is higher due to the presence of double infections, thus the total rate of reported microorganism is 89% in which the highest prevalence was with bacteria (62%), followed by parasites (27 %). The isolated bacteria included, *E. coli* at highest rate (74.84%), followed by *Klebsiella* spp. (15.16%) *Pseudomonas* spp. and *Shigella* spp. at rates of 6.13% and 3.87%, respectively.

Regarding to parasites, all of the detected parasites belonged to protozoa except one case only of *Hymenolepis nana* (0.74%) was reported. *Entamoeba histolytica* was recorded at the highest rate (45.93%), followed by oocysts of *Cryptosporidium* spp. (34.07%), *Blastocysts* spp. (13.33%) and *G. lamblia* (5.93%).

2.3 Results

The detected bacterial species were: *Escherichia coli, Klebsiella* spp., *Pseudomonas* spp. and *Shigella* spp. depending on the colony morphology and biochemical reactions of the cultured stool specimens on different culture media and by using different biochemical tests as shown in Table (1). While parasites were diagnosed depending on the microscopic morphology of their trophozoites/ cysts or eggs.

The types of infection:

Table (4) shows the types of infections, in which single infections were reported at a rate of 61.68% (198/321) of the positive cases, with the highest rate 49.53% (159/198) being with bacteria and only 12.15% (39/198) with parasites. Regarding bacteria *E. coli* was the dominant species reported in 81.13% (129/159) of the cases, while the lowest rate 4.40% (7/159) was with *Shigella* spp. Regarding parasites, oocysts of *Cryptosporidium* spp. was dominant, since it was reported in 61.54% (24/39) cases of single infections, while the lowest rate 2.56% (1/39) was with *G. lamblia*.

As regards to mixed infections, they were reported at a rate of 38.32% (123/321), in 67.48% (83/123), a combination between bacteria and parasites was detected with *E. coli* and *E. histolytica* being the dominant species reported in 33.33% of the cases. Followed by two species of bacteria at a rate of 27.64% (34/123), with *E. coli* as a dominant species, and only 4.88% (6/123) of the cases were with two

species of parasites with the highest rates of *E. histolytica* and oocysts of *Cryptosporidium* spp.

Relationship between the rates of infection with gender and age

The distribution of enteric microorganisms (bacteria and parasites) among both gender and different ages is shown in Table (5). It is evident from the table that the total rate in males was higher than that of females (59.19% vs 40.81%) with the highest (77.89%) being among the age group 6 months to 2 years.

Regarding to the relationship between rate of infection with each gender and age. In males the highest rate was among the age group 6 months to 2 years (62.16%), while in females the highest rate was among the age group 9-12 (51.16%). Statistically the differences between rates of infection with gender and age were non-significant (P = 0.294).

Table (4): Showing types of infections.

Single infection bacteria	**No. of infected**	**%**
E. coli	129	81.13
Klebsiella spp.	9	5.66
Pseudomonas spp.	14	8.81
Shigella spp.	7	4.40
Sub-total	**159**	**49.53**
Single infection Parasites	**No. of infected**	**%**
E. histolytica	2	5.13
Cryptosporidium spp.	24	61.54
Blastocysts spp.	12	30.77
G. lamblia	1	2.56
Sub-total	**39**	**12.15**
Total number of single infections	**198**	**61.68**
Mixed infections		
Bacteria+ Parasites	**No. of infected**	**%**
E. coli+ *E. histolytica*	41	33.33
Klebsiella spp.+ *E. histolytica*	9	7.32
Shigella spp.+ *E. histolytica*	5	4.07
E. coli + *Cryptosporidium* spp.	17	13.82
E. coli + *Blastocysts* spp.	6	4.88
E. coli + *G. lamblia*	5	4.07
Sub-total	**83**	**67.48**
Bacteria+ Bacteria	**No. of infected**	**%**
E. coli+ *Klebsiella* spp.	29	23.58
E. coli+ *Pseudomonas* spp.	5	4.07
Sub-total	**34**	**27.64**
Parasite+ Parasite	**No. of infected**	**%**
E. histolytica + *Cryptosporidium* spp.	4	3.25
Cryptosporidium spp.+ *G. lamblia*	1	0.81
E. histolytica + *G. lamblia*	1	0.81
Sub-total	**6**	**4.88**
Total number of double infections	**123**	**38.32**

Table (5): Show the relationship between both genders and different age groups

Age groups (years)	**No. of examined samples**	**No. of Infected**		**Gender**			
				Males		**Females**	
		No.	**%**	**No.**	**%**	**No.**	**%**
6 months to 2	190	148	77.89	92	62.16	56	37.84
>2-5	124	75	60.48	46	61.33	29	38.67
>5-8	98	55	56.12	31	56.36	24	43.64
>8-12	88	43	48.86	21	48.84	22	51.16
Total	500	321	64.2	190	59.19	131	40.81
	X^2= 2.449		P value= 0.294				

3. Discussion

The source of drinking water and application of hygienic conditions are very significant factors for human health, high infection rates may be attributed to the use of contaminated water (Personal observations during the course of this research). The absence of potable water and inadequate fecal disposal contaminates groundwater, particularly in the absence of water filtering or purification systems, and this might facilitate the spread of certain intestinal bacteria and parasites among the community especially children (Hadi, 2010 and Brown *et al*., 2013).

In the current study in general a high rate (64.2%) of infection with both bacteria and parasites were reported among examined stool specimens. Due to the presences of mixed infections, the rate with bacteria was 62% and that for parasites was 27% raising the infection rate to 89%.

Escherichia coli and *E. histolytica* were the most dominant species reported at rates of 74.84% and 45.93%, respectively. In a previous study in Zakho city, Mero *et al*. (2015) reported slightly lower rate (55.32%) in infants and children of both genders and various ages, this indicate that the sanitary conditions were not improved from that time up to date. Furthermore, in previous study also bacteria and parasites were the dominant agents reported at equal

rates (57.33% and 57.0%), but in the recent study the rate of infectious bacteria was higher than that of parasites. In related research conducted in Baghdad, a similar prevalence of bacteria (58.33%) was found among children (Al-Bayatti *et al.,* 2010). Reports of similar findings in studies performed in other countries, in Bangladesh, (Rashedul, 2011) and in New Zealand (McAuliffe *et al.,* 2013), both authors observed several pathogens in fecal samples, including *E. coli*, parasites, and Rota virus. On the other hand, in Libya, the most often identified pathogens were norovirus, rotavirus, and diarrheagenic *E. coli* (Rahouma *et al.,* 2011). A higher prevalence of bacteria (87%) among children was recorded by Kilic *et al.* (2007) in Gaziantep (Turkey), Badry *et al.* (2014) in Duhok province and AlKhateeb (2008) in Kut city; they recorded bacterial prevalence at 87%, 81.6% and 95%, respectively. This high rate of infections many be related to many factors for instance low parental education, poor health hygiene, inadequate toilet training, lack of exclusive breastfeeding, artificial nourishment, low socioeconomic status, waste disposal, water resources, overcrowding, and environmental conditions (Hellard *et al*., 2000).

Escherichia coli and *Entamoeba histolytica* were the most frequent microorganisms reported in the current study. Similarly, in Duhok province Badry *et al*. (2014) and in Zakho city, Mero *et al.* (2015), both of them found *E. coli* and *E. histolytica* were the most common types of microorganisms with infection rates of 58.43% and 62.5%, for *E. coli* and 25.67% and 46.19%, for *E. histoyltica,* respectively. In Erbil, Al Sorchee *et al.* (2013) found a considerable rates of *E. coli and histolytica* infection among children (35% and 16.4%), respectively. In Saudi Arabia, Al-Shammari *et al.* (2001) stated that *E. histolytica* was the common parasites prevalent in this country 30%, and other parasites were reported at lower rate in his study such as *Entamoeba coli* (10.4%), *A. lumbricoides* (5.5%), *H. nana* (5.4%) and (8.1%) other parasitic infections.

In the current study, other bacterial spp. reported at somewhat high rates included *Klebsiella* spp. and *Pseudomonas* spp. at rates of 15.16% and 6.13%, respectively. In other studies, *Klebsiella* spp. have been reported at high rates (20.12% and 34.01%, while the rates of *Pseudomonas* spp. were at lower rates as, 0.77%, and 2.03%, respectively (Badry *et al*., 2014 and Mero *et al*., 2015).

In the present study, oocysts of *Cryptosporidium* spp. were the second common type among the parasites with infection rate of 34.07% (46 samples out of 135 Positive). In Duhok province Hasan (2020) also, found *Cryptosporidium* spp. oocysts as the most prevalent protozoa with infection rate of 46.62%. Similarly, in Kut city/Iraq Rahi *et al*. (2013) reported a high rate of 33.8% with *Cryptosporidium* spp. oocysts.

On the other hand, lower rates with *Cryptosporidium* oocyst were reported by Al-Alousi and Mahmood (2012), in Mosul, which was 18.9%.

In the present study *Blastocystis* spp. was the third most prevalent type among the parasitic agents with an infection rate of 13.33%. Higher and lower rates with this protozoan have been reported in Iraq and worldwide, such as, Al-Sheikly (2002) and Raof and Abdul-Rahman (2011) in Baghdad city reported infection rates of 21.36% and 24.6%, respectively. In Naples city, Italy, Gualdieri *et al*. (2011) reported a rate of 52.7% among immigrants. On the other hand, lower rates (6%, and 12.2%) were reported by Al-Ougelli (2007) in Baghdad and Ozçakir *et al*. (2007) in Turkey.

Low rate (5.93%) was reported with *G. lamblia.* This parasite has been reported in Duhok province in previous studies even at lower rates by Badry *et al*. (2014) and Hasan (2020) which were 2.1% and 3%, respectively. While in Saudi Arabia, Al-Shammari *et al*. (2001) reported *G. lamblia* at a rate of 37.3% and even a much higher rate (42. 10%) with *G. lamblia* have been reported in Zakho city among infants and

children (Mero *et al*., 2015). The low rate with this parasite among studied population in the present study may be attributed to improvement of the watery quality, because nowadays most people drink bottled water, and *G. lamblia* cysts are mainly transmitted via drinking contaminated water (Personal observation).

In current study only one case of infection with *H. nana*, was recorded representing 0.74%. Similarly, Badry *et al.* (2014) in Duhok province, Mero *et al*. (2015) in Zakho city and Al-Sorchee *et al*. (2013) in Erbil city, all of them reported very low rates with this parasite, which were 0.2, 0.87%, and 0.4%, respectively.

The highest rate of infections with *E. histolytica* and *Cryptosporidium* spp. oocysts may be due to the contribution of many factors such as eating contaminated food, vegetables and raw fruits which washed with unclean water containing infective cysts. In addition to eating street food exposed to flies and other insect that act as mechanical vectors for transmitting infectious agents (El-Sherbini and Gneidy, 2012).

Single infection with one type of microorganisms was reported in 61.68% (198/321) of the positive cases, with the highest rate 49.53% (159/198) being with bacteria and only 12.15% (39/198) with parasites, *E. coli* was the dominant bacterial, species reported in 81.13% (129/159) of the cases, while the oocyst of *Cryptosporidium* spp. was the dominant protozoan, since it was reported in 61.54% (24/39) cases of single infections. Somewhat, close rate (33.25%) of single infection with parasites has been reported by Hasan (2020) in Duhok province. Also, Jameel and Eassa (2021) in Duhok city, detected single infection with intestinal parasites at a higher rate than the double and triple infection (77.1, 22.32 and 0.575%), respectively. In Libya, Rahouma *et al*. (2011) found 37.2% of single infection with microorganism more than the co-infection which was lower (13.8%) in their study.

The mixed infections with two types of microorganisms were documented in 38.32% of positive samples. Similarly, in Duhok province Badry *et al*. (2014) and in Zakho city Mero *et al.* (2015), both of them reported mixed infection at rates of 24.71% and 37.36%, respectively. In Kirkuk, a comparable proportion of mixed prevalence, predominantly with bacteria and parasites, has been recorded among children. (Ali *et al*., 2009) which was 38.34%. Additionally, mixed infections were reported at fewer rates in other surveys including those by Al-Sorchee *et al.* (2013) in Erbil and El-Sheikh and El-Assouli (2001) in Saudi Arabia with rates of 4.4% and 3.1%, respectively.

According to the gender and age, the total rate of infected males was higher than females (59.19% vs 40.81%). Similarly, in Duhok province, Badry *et al*. (2014) and in Zakho city Mero *et al.* (2015), showed that the frequency of infected males were more than females (53.26 and 46.74%) and (55.32 and 44.67%), respectively. In Erbil city, Al Sorchee *et al*. (2013) also, reported higher frequency of infection in males compared to females (64.2% vs 35.8%). Most previous studies mentioned that the high rate of infection in males might be due to the outdoor activities of males in addition, they are in more contact with environmental conditions and direct contacts with the source of the infection than females (Jameel and Eassa, 2021). Regarding to the relationship between infection rate with gender and age. Males at ages of >6 months-2 and 3 -5 years were mostly infected (77.87% and 60.48%), while females at ages of 6-8 and 9-12 years were the most infected (48.64% and 51.16%), respectively. Female children are more likely to consume homemade food and less likely to play outdoors. Furthermore, the educational level of mothers and their profession, educational level of parents, dietary knowledge, all these factors have great impact in the increased prevalence of

microorganisms (Alaa *et al*., 2014).

A slight drop in the incidence rate among older children might be explained by the fact that most intestinal microorganisms elicit at least partial protection against infection (Sule *et al*., 2011).

In conclusion, the current study showed that high rates of diarrhea in infants and children are due to enteric bacteria and protozoa and their rates were somewhat comparable to previous surveys performed in this area and other parts of the country. These high infection rates are of public health importance. Therefore, health authorities must pay more attention to this situation by improving the infection control processes, performing screening tests for children at pre and primary school levels, implanting health education programs among the community and a warring them about the effect of these microorganism on the child heath, development and learning capability.

4. Declarations

This study is an original study, has not been before submitted, accepted or published in any scientific journals.

6. Data Availability

All data present in this manuscript are available freely.

7. Acknowledgments

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8. Conflicts of Interest

We declare that there is no any conflict of interest in this study.

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