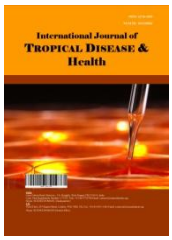

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Prevalence of Some Diarrhea Pathogens among under 5, in Wad Medani Pediatric Teaching Hospital, Gezira State, Sudan

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Infections of gastrointestinal tract are the most frequent diseases worldwide. Infectious diarrhea is a major public health problem and remains one of the most important causes of morbidity and mortality among infants and children especially in developing countries. The symptoms of gastroenteritis include diarrhea and vomiting which may lead to severe dehydration and even death.

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The aim of this study was to identify some diarrheal pathogen among under five children attending to wad Medani Pediatric Teaching hospital during January 2021 to march 2022. Total of 384 stool samples were collected from children suffering from diarrhea and were tested using culture with biochemical test, direct examination by wet preparation and staining techniques. Moreover, screen *Rotavirus* by Ag ICT were used to determine their prevalence. The data were collected using questionnaire and analyzed using Statistical Package for the Social Sciences (SPSS). The results showed that the prevalence of pathogens were 196 samples (51%). The prevalence of pathogens showed that (29.6 %) was *Giardia lamblia*, (19.9%) was *Cryptosporidium*, (13.3%) was *Rotaviruses*, (12.2%) was *Escherichia coli*,(9.7%) was *Schigella species*,(8.7%) was *Entamoeba histolytica* and (6.6%)was *Salmonella species*. From those infected patients,(56.1%) had fever, (76%) had vomiting. Also, (39.3%) were resides in urban while(60.7%) resides in rural situation. About (42.9 %)had severe dehydration, (60%)acute diarrhea and (40%) chronic diarrhea , (87.8%) of children was take Rota vaccine and (70%) of mother not use soap to cleaning the children and think diarrhea not infectious disease it cause by teething. In conclusion most of diarrhea cause by parasitic infections mostly in poor family that not able to provide safety water and sanitation and low education of mother also some children suffer from malnutrition which decrease immunity and give chance to infection (*Cryptosporidium*).
In Recommendation, health education about diarrhea disease in child important for mother to prevention, attention should be Payed proper to hygienic measure such as hand washing, safe disposal of feces and disinfection of contaminated surface to reduce the risk of infections agents transmission.

Keywords: Diarrhea; rotavirus; parasitic and bacterial infections; central sudan.

1. INTRODUCTION

Diarrhea disease remain one of the leading cause of preventable death in developing countries especially among children under five years of age, diarrhea is common in the developing countries especially in areas with poor hygiene and sanitation and with limited access to save water [1]. Neonatal diarrhea classified to acute diarrhea, Which more severe and more common, example of organism cause acute diarrhea *shigella spp* and chronic diarrhea more than 14 days which less sever, less common, example of organism cause chronic diarrhea *salmonella spp* [2]. Diarrhea is defined an intestinal disorder characterized by abnormal fluidity and frequency of faces, at least three times with in 24 hour period (Kenneth et al. 2004). About 80 of death due to diarrhea occur in the first two years of life the main cause of death from acute diarrhea is dehydration which result from the loss of fluid and electrolytes in diarrhea stool [3]. The lack of facilities in many routine and teaching laboratories in Sudan lead physicians and microbiologists excluding important etiological agents of diarrhea, Although some of these agents have bad consequences and may lead to Life threatening diseases eg: haemolytic uraemic syndrome that is cause by *Enterohaemorrhgic E. coli* [4]. The onset of diarrhea in children may rapidly lead to life threatening dehydration and malnutrition so clinical and epidemiological studies defining the etiology are needed in order to improve diagnostic and therapeutic approaches for the management of diarrhea in children [5]. Diarrhea in infant and children is always serious because they have such small extra cellular fluid (ECF) reserved than adult. The sudden losses of water exhaust the supply quickly, the loss of extracellular sodium lead to decrease in plasma volume and possible circulatory circulatory collapse, renal failure could result with irreversible acidosis and death [6]. Organisms that cause diarrhea including bacteria eg: *shgilla spp, salmonella spp, vibrio cholera, campylobacter* Virus eg.: *Rotavirus* also parasites eg: *Entamoeba histolytica and cryptosporidium* [7]. These organisms are transmitted from faces of one individual to the mouth of another, a rout termed fecal oral transmission. However they differ in exact rout of entry from stool to mouth and in the infectious dose needed to cause the illness (Sher Chand et al. 2009). The epidemiology of *diarrheagenic E coli*: In Burkina Faso, Bonkaaungou et al [8] investigated occurrence of *diarrhea E coli* in stool samples from 658 children with diarrhea, diarrhea E coli were isolated from 214 samples (Bonlounou et al. 2012). Epidemiology of *shigella spp* in Sudan [9] Musa Abdalla et al studied *shigella* gastroenteritis in Khartoum state, he reported that 16 *shigella species* were isolated from 100 (16%) stool specimens [9]. In the study

done by Jafari in the Tehran, Iran [10] in 808 stool samples of patients with acute diarrhea, found 369 (45%) were bacterial pathogens result showed *shigella species* were 155 (45.6%), *diarrheagenic E. coli* were 143 (38.8) , *salmonella spp* were 51(13.8%)and *campylobacter spp* were 20 (4.5%) [10]. Ling and Cheng et al. [11] , studied the role of enteric pathogen in children in Hong Kong Gastroentric *salmonella* were the most common pathogens (45%) followed by *Rotavirus* (34%) and *E coli* (1%) [11]. The etiology of diarrhea in children less than 5 years old in Bangkok, Thailand was determined by Varvithya. w. et al [12] the following isolates were reported: *non typhoid salmonella spp* (13%) *Cambylobacter, Jejuni* (12%) Rotavirus (12%) *E coli* (8%) and *shigella spp* (6%) [12]. This study in Central Sudan, aimed to isolate and identify the common bacteria associated diarrhea (*Shigella spp*, *Salmonella spp and E. coli)* and identify some parasites that cause diarrhea (*E. histilytica, Giardia lamblia and cryptosporidium*) and using Ag ICT to determine *Rotavirus* microorganism.

2. METHODOLOGY

2.1 Study Design and Study Area

This prospective descriptive study to identify Diarrhea in Patients in Wad Medani Pediatric Teaching Hospital Gezira State The Study conducted during the period (Jan 2021 - march 2022).

2.2 Study Population and Subject Selection

Sample size: $n = \frac{z2 \times p2 \times q2}{d2} = \frac{1.96 \times 1.96 \times 0.5 \times 0.5}{0.05 \times 0.05} =$ 384

n : sample size
p : prevalence of disease
q : 1-p
d : permeable error

384 diarrhea sample were taken by simple random sampling from children under 5 years old attending Wad Medani Pediatric teaching hospital, (Gezira State) with diarrhea during study period (2021 - 2022) all tribes and both gender were included (Child above 5 years were excluded) also ethical permission was take from the ministry of health and from the faculty of medical laboratory science, permission from the head director of the hospital and consent from patient's family.

2.3 Data Collection and Analysis

- A well designed questionnaire includes personal information and clinical data all children under 5 in the word of diarrhea in the Wad Medani Pediatric Teaching Hospital during the study period were included in the study (all tribe and both sex included) (children above five years were excluded).laboratory tests and data were analyzed by Statistical Package for the Social Sciences SPSS (version 20).

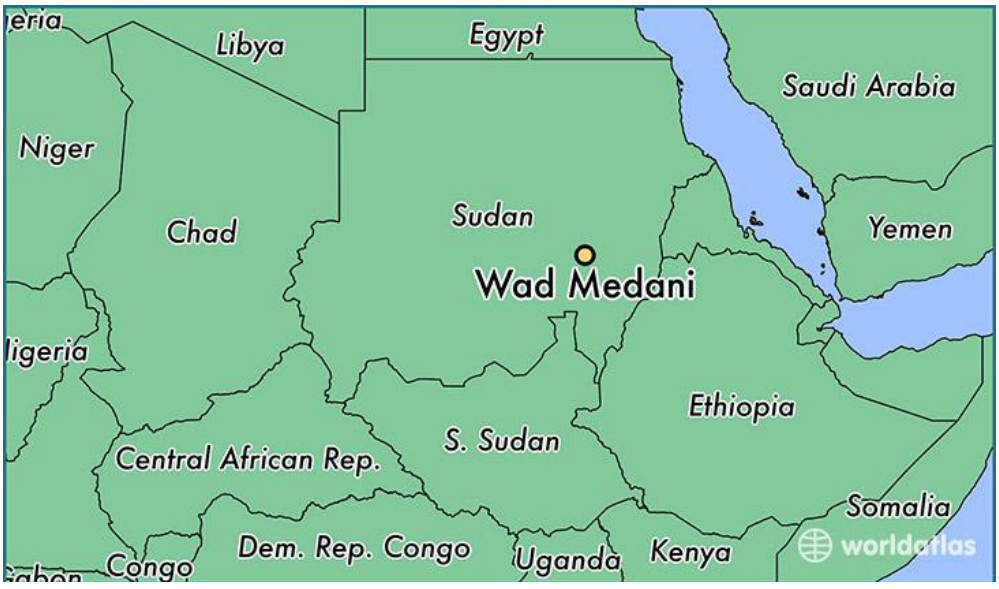

Fig. 1. Location of Wad Medani city, Gezira State, Sudan

2.4 Diagnosis of Organisms Cause Diarrhea

Diarrhea samples were collected in sterile wide stool containers contain spoon. Any diarrhea sample divided into three containers first one culture in maconkey and blood agar to identify bacteria, second for wet and stain preparation (normal saline, iodine and modified ZN stain) to identify parasite and third for *Rotavirus* screen use Ag ICT.

2.4.1 Identify of bacteria

On day one culture and isolation of bacteria by using macconkey agar media and XLD media (differentiate between lactose and non lactose fermenting bacteria) then incubate at 37 c over night.

Identification of bacteria on day 2 by gram stain and biochemical tests.

Gram Stain:

1- Preparation of smear

- Slide was passed on flame three times to remove fat.
- Sterile wire loop was used to take small portion of growth on the culture media and smear made by dissolving in the drop of normal saline until it become homogenous and allow the smear to dry by air.
- The smear was fixed by passing in to the flam three times.

2- Then this smear stained by the Gram Stain and Biochemical test was performed (Urease Test, Indole Test, Citrate utilization test, Motility test and Kiligler iron agar).

And on day 3, the above tests results were read and causative agents were identified:

E.coli : Indole (+ve), citrate(-ve) urease (-ve), Motility (+ve), H_2s production(-ve) No blacking in KIA, yellow slope and yellow butt (*E.coli* Ferment glucose and lactose).

Shigella spp :urease (-ve) Motility (-ve), citrate(-ve) , Not produce H_2s (No blacking) in KIA yellow butt, red slope because *Shigella spp* ferment glucose and Non ferment lactose.

Salmonella spp: urease (-ve), citrate (-ve), Motility (+ve) Indole (-ve), H_2s production (+ve) except paratyphi A which is (-ve) In KIA Red slop, yellow but because ferment glucose and non ferment lactose.

2.4.2 Identification of parasite

Wet preparation using physiological saline:

Using piece of stick place small amount of diarrhea specimen to the drop of saline mix and cover with cover glass and using tissue press gently on the cover glass to make a thin preparation and Examine immediately by microscope using 10 x and 40 x objective to identify motile trophozoit of *Giardia lamblia* or *E.histolytica* and also see the cyst of *Giardia .lamblia* and *E.histolytica* and oocyst of *C. parvum*.

Iodine preparation:

Drop of iodine in slide and add small amount of diarrhea and mix and cover using cover glass to make thin preparation and examine by microscope using 10x and 40x to see Trophozit and cyst of *E.histolytica* and *Giardia.lamblia*.

***Trophozit* of *E. histolytica*:** size about 25x 20Mm, active amoeboid movement in fresh specimen, single nucleus which has central karyosome , may contain ingested red cell.

Cyst of *E. histolytica*: Round measuring 10-15Mm

- Contain 1,2 or 4 nuclei with a central karyosome.

Trophozoit of *Giardia lamblia* :

- Small pear shaped flagellate with a rapid motility like a falling leaf.
- Measures 12-15x5-9Mm has a large sucking disc and it has four pairs of flagella, two axonemes and two nuclei, a single or two median bodies are present.

Cyst of *Giardia lamblia* :

- small and oval measuring 18-12x about 6 Mm, Four nuclei, axonemes, median bodies and remain of flagella.

Modified Ziel Neelsen Technique:

The smear were prepared from sediments of centrifuged stool sampl , air dried and fixed with

absolute methanol for 5 minutes . The smears were stained with carbol –fuchsin for 30 minutes and washed with tape water. The slides were decolorized in acid alcohol for 2 minutes and were counter stained with methylene blue for another 2 minutes finally the stained smear were examined using oil immersion objective to detect ooysts of *Cryptosporidium parvum*. (cheesbrougsh, 1987).

Oocyst of *Cryptosporidium*:

Small, round oval, pink red stained bodies measuring 4-6 Mm (acid fast).

2. 3 Identification of Rotavirus Using ICT

Rotavirus Combo Rapid Test Cassette (Feces) Package Insert (REF IMVD-645 | English).

Rapid Test (Immunochromatography Test (ICT) Relative sensitivity (97.3%), Relative specificity (97.1%), Relative accuracy (97.2%).

After preparation of sample 80ML were addedx to (ICT) strip after migration of sample the reaction was observed and the result was determined (positive 2 lines negative 1 line).

3. RESULTS

The Study was conducted during the period from January 2021 to March 2022 to identify Pathogens that cause diarrhea among under 5 children in Wad Medani pediatric teaching Hospital.

From the total 384 stool (samples, identification of causative agents was possible in 196 specimens (51%) according economic status of family of child with diarrhea mostly poor income (65.3%) and medium (29.1%) and very few family rich (5.6%) (Table 4a).

Giardia lamblia was the predominant microorganism (29.6%) followed by *cryptosporidium* (19.9%), *Rota virus* (13.3%), *E. coli* (12.2%), *Shigella spp* (9.7%), *Entamoeba Histolytic* (8.7%) and *salmonella spp* (6.6) (Fig. 2) the study found there is No difference between prevalence of diarrhea between male (52%) and female (48%) (Fig. 3).

Most of those who developed diarrhea were within age groups (less than one year) (60.7%), 1-2 years (26.5%), 2-3 years (7.7%), and 3-4years (5.1%) (infection decrease with increase of age) (Table 4b) and most of Patient in Rural (60.7%) and less in Urban (39.3%) Fig. 4.

In this study (42.9) of patient suffering from severe dehydration, (56%) had Fever and (76%) had vomiting (Table 4c).

(59.7%) of child had Acute diarrhea and (40.3%) had chronic diarrhea, (29.6%) of child cleaning with soap by his mother and(70.4%) of child not cleaning with soap (87.8%) of children were take Rota vaccine while(12.2%) of children were not take Rota vaccine (Table 4d).

Parasitic infection was Found in 59.2% of Specimen, and culture positive in (29.1%) (Table 4e).

Table 4a. Distribution of study population according to causative agent and economic status of child family

		Frequency	Percent
Valid	Positive	196	51
	Negative	188	49
	Total	384	100
Income	Rich	11	5.6
	Medium	57	29.1
	Poor	128	65.3
	Total	196	100.0

Table 4b. Distribution of study population according to Age

		Frequency	Percent
Valid	Up to 1Year	119	60.7
	1 - 2Year	52	26.5
	2 - 3Year	15	7.7
	3 - 4Year	10	5.1
	Total	196	100.0

Table 4c. Distribution of study population according to Severe Dehydration, appearance of fever and vomiting

		Frequency	Percent
Sever Dehydration	Yes	84	42.9
	No	112	57.1
	Total	196	100.0
Appearance of fever	Yes	110	56.1
	No	86	43.9
	Total	196	100.0
Vomiting	Yes	149	76.0
	No	47	24.0
	Total	196	100.0

Table 4d. Distribution of study population according to Type of Diarrhea and Cleaning of the child with soap by his mother and did Child take Rota vaccine

		Frequency	Percent
Type of diarrhea	Acute	117	59.7
	Chronic	79	40.3
	Total	196	100.0
Cleaning of the child with soap by his mother	Yes	58	29.6
	No	138	70.4
	Total	196	100.0
Did Child take Rota vaccine	Yes	172	87.8
	No	24	12.2
	Total	196	100.0

Table 4e. lab result(a)Direct microscopy wet and stain preparation for parasitic infection and culture for bacteria

		Frequency	Percent
Lab result (wet and stain preparation) for parasite infection	Positive	116	59.2
	Negative	80	40.8
	Total	196	100.0
Lab result culture for bacteria	Positive	57	29.1
	Negative	139	70.9
	Total	196	100.0

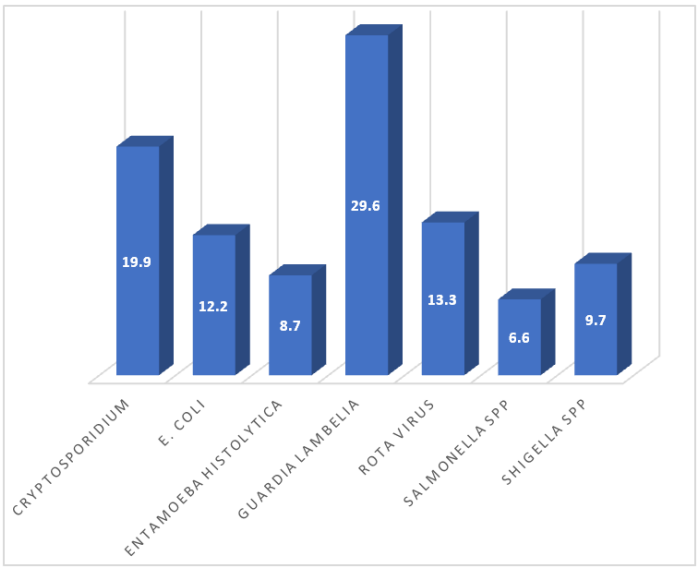

Fig. 2. Causative agent of diarrhea

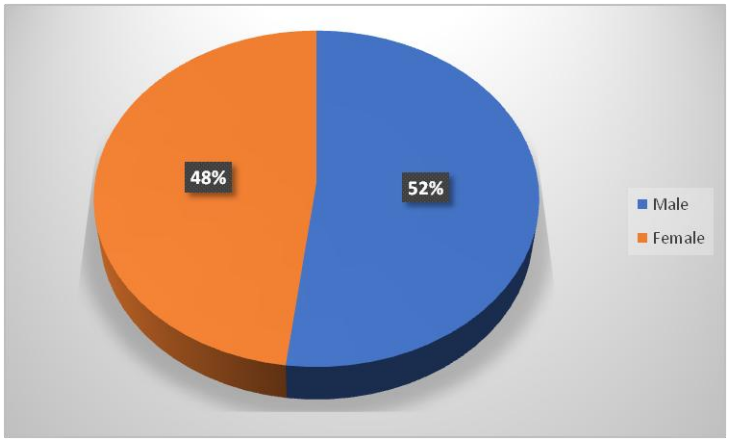

Fig. 3. Distribution of study population according to Gender

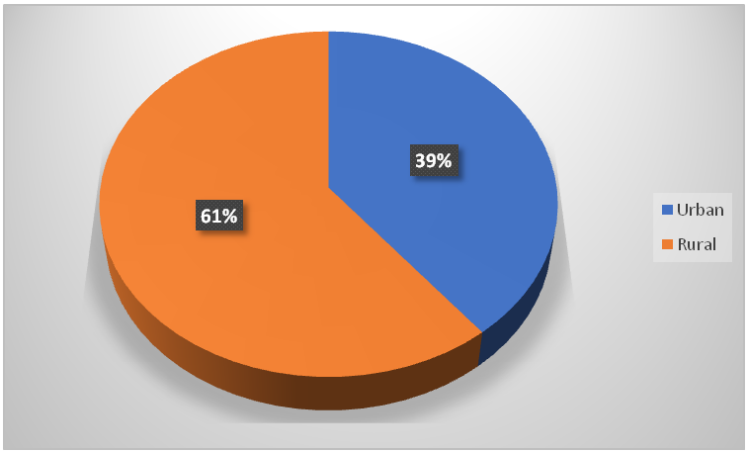

Fig. 4. Distribution of study population according to Residence

4. DISCUSSION

Diarrhea diseases remain a serious public health and causes morbidity and mortality worldwide.

Although diarrhea in children cannot be completely eliminated, a reduction of infection rate to a minimal level could have significant benefits by reducing diarrhea morbidity and mortality.

In the present study ICT and wet preparation and stain preparation and cultural techniques and standard biochemical tests were used for identification of parasite, Rotavirus and bacterial infections.

In the present study found causative agent in (51%) of population (49%) of study population not found causative agent may be the diarrhea due to antimicrobial associated diarrhea, or food allergy (eg: cow milk allergy) or congenital diarrheal disorders (e.g.: Glucose galactose - Malabsorption, congenital chloride diarrhea) or Metabolic disorder or gastrointestinal infection by organisms not contained in the study e.g.: *Toga virus, yersinia, vibrio*, *campylobacter*, different warms and other.

The study by Passariello A, et al. [13] show that etiology defined in (74.3%) of cases and this result disagree with this present study which identified causative agents in (51%) of cases.

This study found the *Salmonella spp* (6.6%), This isolate rate can be considered less in comparison with the isolation rate reported by ling and change et al 1993 in Hongkong in which *Salmonella spp* (45%) and also varvithya. w. et al [12] in Bangkok Thailand in which *Salmonella spp* (13%) These rate reported may be not a true reflection due to nature of most Sudanese who don't usually seek medical attention or

investigate their stool when the child suffering from diarrhea.

Shigella spp are important pathogens that causes bloody diarrhea in developing countries .In the present study the frequancy of *Shigella spp* was (9.7%) this isolate rate consider less frequancy in comparison with isolate reported by Musa Abdalla et al study in kartoum state 2002 in which *Shigella spp* (16%) and also study done by Jafari in Tehran [10] in which *Shigella spp* (45%), this may be due to different geographical distribution of bacteria, different environmental factors and different antimicrobial used.

In the present study prevalence of diarrhea *E. coli* less in comparison with study conducted by Bonkaugou et al. (2011) in Burkina Faso in which isolate 214 diarrhea E.coli from 658 children with diarrhea, this may be due to different hygienic measures, environmental factors and different use of disinfectants.

My study found most causative agents Giardia and cryptosporidium and this disagree with study conducted by Hiroyuki Hanai in [14] diarrheal cases in japan 2012 which found most causative agent *E. coli.*

In this present study parasitic infection (*E. histilytica, Giardia and cryptosporidium*) frequency 59.2% which is high percentage, due to low quality water and sanitation and low mother education about diarrhea and important of cleaning child with soap (70%) of mother not use soap and think diarrhea not infections disease, it cause by teething due to low level of education.

In this study *Giardia lamblia* (29%), *E. histilytica* (8.7%) and considerable number of *cryptosporidium* (19.9%) (Some child had malnutrient).

In this study there is No difference between male and female.

This disagree with study conducted by Roy et al 2012 which found prevalence of Rotavirus infection more in males due to different environmental and geographical condition.

The rate of diarrhea in Rural (60.7%) more than rate of diarrhea in urban(39.3%) due to different environment and different economic condition.

In this study (42.9%) of child suffer from severe dehydration when attending the hospital and need Intravenous rehydration and (57%) of children haven't or have low degree of dehydration and need oral rehydration solution.

In this Present study found Prevalence of Rotavirus (13-3%) which is less than that reported by ling and cheng *et al* which found (34%) detection of Rotavirus and this attributed to that the Rotavirus vaccine is not applied in routine vaccinations Also Result of our study less that study conducted by WHO in Sudan during Jan-Des 2009 which found. Rotavirus causes (42%) of childhood diarrhea hospitalization (WHO 2010). And also less than study conducted by (AbELAMeeren 2006) in Gaza Palestin in which Rotavirus (28%).

5. CONCLUSION

Gastroenteritis is the most common worldwide disease associate with significant morbidity and mortality among children under five years of age, This study showed (43%) suffer from dehydration,(59.7%) acute diarrhea and(40.3%) chronic diarrhea , (76%) developed vomiting and (56%) developed fever. Referring to age (60.7%) less than one year,(39.3%) were urban and (60.7%) were rural. and most of diarrhea in Autumn. Most common causes of diarrhea were Giardia lambelia (29.6%) and cryptosporidium (19.9%) This study showed decrease in the prevalence of Rota virus from (28%) in 2015 to (13.3%) in (2021-2022).

6. LIMITATION OF THE STUDY

Study identify some but not all diarrhea pathogens, study determined prevalence of three parasites only (*E. histilytica, Giardia and cryptosporidium*) not all parasites that cause diarrhea. Also study isolate and identify certain bacteria that cause diarrhea (*Shigella spp*, *salmonella spp, E. coli)* not all bacteria that cause diarrhea to children also must use molecular methods example PCR for confirm of *Rotavirus* and other diarrhea organisms because it more sensitive, specific and accurate (gold method) but very expensive and Time consuming and not available reagents and need expertise Person and expensive PCR machine.

7. RISK FACTORS OF DIARRHEA

The most risk factors in under 5 children were small age (less than one year) the infection decreases with increase of age, also poor income, vomiting, low health education of mothers and not use soap for clinging of child (bad hygiene) and rural residence [15,16].

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the IRB of the Faculty of Medical Laboratory Science, University of Gezira. All participants signed a standard informed consent.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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