ORIGINAL ARTICLE

Diagnosis and Management of Dysentery in Children Attending National Hepatology and Tropical Medicine Research Institute

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strategies about risk factors are needed to develop recent controlling methods.

ABSTRACT

INTRODUCTION

Dysentery, is an gastrointestinal disorders defined as invasive diarrhea with visible blood in one or more stool, usually colon inflammation occurs, many infectious pathogens are responsible as protozoa, parasitic worms, bacteria and viruses, it may also result from intestinal chemical irritation ^{1,2}. Infections occur through ingestion of contaminated food or water, oral contact with contaminated objects or hands. Pathogens reach the large intestine, each has its own mechanism and pathogenesis, the final result is damage of the intestinal linings and inflammatory immune responses³. Characteristic features include abdominal pain / cramps, tenesmus, frequent passage of watery diarrhea or stool containing blood/mucous⁴. Dysentery is often associated with high morbidity and mortality rate ,globally affects young children in developing countries, poor living status, overcrowded houses, inadequate sanitation and poor hygienic conditions found to be associated with increased risk of acquiring dysentery⁵. There are two main types of dysentery; intestinal amoebiasis and

bacillary dysentery, mild bacillary dysentery may last for 4 to 8 days, severe cases may last for 3 to 6 weeks. Amoebic dysentery starts gradually and usually lasts about 2 weeks⁶. Most cases of amoebic or bacterial dysentery could subside within 10 days, and full recovery within two to four weeks after beginning proper treatment 7 , the prognosis varies according to the patient's immune status and disease severity⁸, although dysentery is preventable and treatable disease, 1 billion childe are presented with diarrhea and around 525000 children < 5 years old died yearly⁹. Dysentery has severe nutritional consequences for children including protein -losing enteropathy and depressed / slow growth patterns in children that may persist up to one year post - infection¹⁰. Hand washing / food safety are important measures in prevention especially in areas and subjects of high risk¹¹. Diagnosis is made by taking personal history, brief clinical examination, dysentery should not be confused with hematochezia (passage of fresh blood through the anus in or with stools) and laboratory examinations. Management is maintained by using rehydration therapy (oral/intravenous), antiparasitic,

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antiviral or antimicrobial therapy. A combination of drugs may be necessary when laboratory services are not available ¹². Hence, the current study was conducted to evaluate the most common enteric pathogens causing dysentery in children and the most associated risk factors, aiming to rapid, proper diagnosis and treatment to eliminate the disease.

METHODOLOGY

The current study was conducted on 180 child patients aged from1-18 years old, attending NHTNRI from October 2019 to January 2020, patients were presented with symptoms/signs of dysentery (mainly diarrhea), with no prior antibiotics, antiparasitic, antiviral chemotherapy or other medical drugs. The study was approved by Ethical committee of the General Organization for Teaching Hospitals and Institutes (GOTHI), written /verbal consent was taken for each patient was taken by parent/parents or guardian.

Each patient was subjected to:

- Questionnaire sheet: Name, age, gender, level of education for parents, socioeconomic status ,source of drinking water, sewage disposal system, complaint, fever, vomiting, type of diarrhea, fecal blood /mucous.
- Physical and Abdominal U.S examinations.

Laboratory investigations:

Type of samples and collections:

Fresh stool sample was collected from each child in a clean, sterile, dry, labeled container, processed and analyzed for:

Parasitological examinations:

- *Macroscopic examination:* The visible fecal blood, mucous, pus, worms and fecal consistency were detected.
- Microscopic examination: Parasitic eggs, larvae, cysts and trophozoites were detected after preparation of the stool samples using direct smear stained (with iodine), Formalin-Ether concentration and modified Ziehl- Neelsen (ZN) methods ¹³.
- Copro-Antigens detection: For Cryptosporidium parvum/Giardia lamblia/Entamoeba using immune-chromatographic quantitative rapid assay, supplied by Combi-RIDA QUICK TEST, N1722 r –biopharma –Germany. Analysis was conducted as described by the manufacture instructions.

Bacterial examinations:

'Standard culture and identification methods were used'.

- Fresh fecal samples were cultured on MacConkey agar to identify gram negative bacilli, Sorbitol MacConkey agar to differentiate between pathogenic and non-pathogenic *E.coli* strains and S.S agar to identify and differentiate *Shigella* from *Salmonella*

strains ,colonies were isolated in pure culture and identified using biochemical reactions and microscopic examination (after Gram staining).

Antimicrobial sensitivity:

The antibiotic sensitivity (to determine the most sensitive antibiotics) of the isolated micro-organisms to different antibiotics was tested using Mueller–Hinton agar and the standard disc diffusion technique of the modified Kirby–Bauer method, as recommended by the Clinical and Laboratory Standards Institute, antibiotic discs were inoculated for each sample on Muller Hinton agar and inoculated at 37 c for 18-24 hours.

Rota virus Antigen Detection: - Using commercially available Latex agglutination test (RIDA –SCREEN, by biopharma- Germany). Analysis was done as described by the manufacture instructions.

Management:

- Pretreatment phase (clinical and laboratory examinations).
- Treatment phase (all patients were treated at homes) by:-
- Fluid replacement and maintenance (using oral rehydration therapy) and Zinc supplements for watery diarrhea.

Antiparasitic therapy:

Metronidazole for giardiasis /amoebiasis, and Praziquantel or Albendazol as broad spectrum anthelminthic

- N.B .Cryptosporidium is a self-limiting.
 - *Antimicrobial therapy* (according to sensitivity test):

Most sensitive antibiotics were ciprofloxacin /Carbapenems for *E.coli* infections, Cephalosporins /Ceftriaxone for *Salmonella* infections, Chloramphenicol/Ciprofloxacin for *Shigella* infections and amikacin/ imipenem for *Klebsiella* infections.

N.B: For Rota virus infection, no specific medication, but it was very important to control dehydration.

- *Supportive care* (*SC*): Includes continued good diet, breast feeding, encourage the child to eat and select his preferred foods.
- Awareness about prevention of infectious diseases, proper hygiene environment, hand washing and nutrition.
- Follow up by clinical/laboratory examination.

Results showed that all patients had completed recovery after following the treatment programs (proved by laboratory and clinical examinations).

Statistical Analysis:

The chi-squared (χ^2) test was used to determine the statistical significance of the data. A *P*-value of <0.05 was considered statistically significant.

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RESULTS

180 children suffered from dysentery aged 1-18 years with mean age of (7.7 ± 3.6) . (61.6%) were >5 years old, (38.4%) were <5 years old either ≤ 2 years (16.7%) (Of them, 53.3% were with breast feeding history), 21.7% 3-5 years; with slightly more distribution in females (51.1%).

73.3% children's parents were of mild to moderate educational level, (21.1%) were illiterate and 5.6% of well education.

The majority have adequate sanitary condition in form of safe water supply (84.4%) and proper sewage disposal (85.0%). (Table 1 & figure 1)

Studied groups	Childr	Children with dysentery		
Characteristics	No. 180			
Age -Range		1-18		
- Mean ±SD9years)		7.7 ±3.6		
	No.	%		
Age groups				
≤2	30	16.7		
3-5	39	21.7		
>5	111	61.6		
Sex : Male	88	48.9		
- Female	92	51.1		
Education level of Parents				
- Illiterate	38	21.1		
- Mild	78	43.3		
- Moderate	54	30.0		
- Well	10	5.6		
Safe water supply				
- Yes	152	84.4		
- No	28	15.6		
Proper sewage disposal				
- Yes	153	85.0		
- No	27	15.0		

Table 1: General profile of studied children



 ≤ 2 years

Among 180 children, 23.3% & 18.3% cases gave history of fever \geq 38.5c, vomiting respectively. 19.4% with watery diarrhea, 46.1% of moderate frequency and 13.9% with dehydration.

Visible fecal blood was 13.9%, 21.7% pus / mucus, 95.6% with normal abdominal U.S.

Rota virus was detected in 11.1%. Bacterial agents were detected in 34.4% mainly due to *E.coli* 42.0% followed by *Salmonella spp.* 25.8%, *Shigella spp.*17.7% and *Klebsiella* 14.5%. Intestinal parasites were detected in 45.0% as 22.2% *Giardia lamblia,* 21.0% *E.histolytica* and *cryptosporidium* 9.9%, *E.vermicularis* 8.6%, *S.mansoni /A. lumbricoides* 4.9%, *Hook worm/H.nana* 3.7%, *H.hetrophyes* 2.4%, *Fasciola* 1.1%. (Table 2, figures 2&3).

Studied groups	Children v N	vith dysentery o. 180
Clinical Characteristics	No.	%
Fever ≥38.5		
-No	138	76.7
-Yes	42	23.3
Vomiting		
-No	147	81.7
-Yes	33	18.3
Dehydration		
-No	155	86.1
-Yes	25	13.9
Watery diarrhea		
-No	145	80.6
-Yes	35	19.4
Frequency of diarrhea		
-Mild	66	36.7
-Moderate	83	46.1
-Sever	31	17.2
Visible fecal blood		
-No	155	86.1
-Yes	25	13.9
Fecal Pus / mucus		
-No	141	78.3
-Yes	39	21.7
Abdominal U.S		
- Normal	172	95.6%
- Hepatomegaly	8	4.4%
Rota virus		
-No	160	88.9
-Yes	20	11.1
Bacteria		
-No	118	65.6
-Yes	62	34.4
Parasites	00	55.0
-No	81	55.0 45.0
-Yes	01	45.0

 Table 2: Clinical profile of studied children



Fig 2: Types of bacteria detected in children



N.B: others included those with mixed parasitic infections.



Fig. 4: Distribution of causative agents.

(22.2%) were without organisms, and (77.8%) with different agents as 35.6% parasites , 21.7% bacterial, 3.9% *Rota virus* parasites with bacteria were 9.4%, parasites with *Rota virus* were 4.4%, Bacteria with *Rota virus* were 1.7%, parasites & bacteria and *Rota virus* were 1.1%.

Studied groups	Negative	Parasites	Bacteria	Rota virus	Parasites &	Parasites &	Bacteria	All	Test of
Items	(40)	64	39	7	Bacteria 17	Rota virus 8	& Rota virus 3	2	sig. &p.
-Age in years									
Mean <u>+</u> SD	6.5 <u>+</u> 2.7	8 <u>+</u> 3.8	8 <u>+</u> 3.6	5.3 <u>+</u> 2.1	8 <u>+</u> 4.1	6.3 <u>+</u> 3.3	10.3 <u>+</u> 4.8	10 <u>+</u> 4.5	Ns
Sex - Boys	21.6%	40.9%	14.8%	5.7%	11.4%	4.5%	0.0%	1.1%	
- Girls	22.8%	30.4%	28.3%	2.2%	7.6%	4.3%	3.3%	1.1%	NS
Insanitary water supply	0.0%	50.0%	17.9%	3.6%	10.7%	7.1%	7.1	3.6%	0.012*
Insanitary sewage disposal	7.4%	48.1%	11.1%	3.7%	11.1%	11.1%	3.7%	3.7%	`NS
Parents education									
- Illiterate	15.8%	28.9%	26.3%	2.6%	10.5%	7.9%	2.6%	5.3%	
- Mild	17.9%	44.9%	19.2%	3.8%	10.3%	2.6%	1.3%	0.0%	NS
- Moderate	29.6%	25.9%	24.1%	5.6%	9.3%	5.6%	0.0	0.0%	
- Well	40.0%	40.0%	10.0%	0.0%	0.0%	0.0%	10.0%	0.0%	
Fever ≥38.5	7.1%	21.4%	23.8%	4.8%	21.4%	14.3%	7.1%	0.0	0.001*
Vomiting	6.1%	30.3%	18.2%	9.1%	15.2%	18.2%	0.0%	3.0%	0.001*
Dehydration	8.0%	36.0%	8.0%	16.0%	12.0%	12.0%	8.0%	0.0%	0.001*
Watery diarrhea	0.0%	25.7%	20.0%	8.6%	14.3%	20.0%	5.7%	5.7%	0.001*
Frequency of diarrhea									
- Mild	31.8%	30.3%	24.2%	0.0%	12.1%	1.5%	0.0%	0.0%	
- Moderate	20.5%	45.8%	22.9%	0.0%	7.2%	0.0%	1.2%	2.4%	0.001*
- Sever	6.5%	19.4%	12.9%	22.6%	9.7%	22.6%	6.5%	0.0%	
^a Visible blood in stool	0.0%	32.0%	28.0%	0.0%	24.0%	12.0%	0.0%	4.0%	0.004*
^a Pus \ mucus in stool	5.1%	35.9%	20.5%	5.1%	15.4%	12.8%	2.6%	2.6%	0.013*
^a Hepatomegaly	0.0%	87.5%	0.0%	0.0%	12.5%	0.0%	0.0%	0.0%	NS

 Table 3: Comparison of general and clinical profile according to causative agents of dysentery

The mean age with different agents were nearly equal. Most boys had parasites(40.9%) followed by bacteria 14.8%, for girls (30.4% & 28.3% respectively). parasitic infection was more among children with insanitary water supply (50.0%), bacteria (17.9%), both infections (10.7%); while ratio among those with insanitary sewage disposal were (48.1%, 11.1% & 11.1% respectively). Among those of illiterate parents had either parasitic 28.9% or bacterial infection 26.3% or both 10.5%; most of mild education had parasitic 44.9%, bacteria 19.2%, among moderate education mostly had no organisms 29.6% or 25.9% parasites, 24.1% bacteria, among well-educated parents 40 % had no agents, 10% had bacterial and 40 % had parasitic infection. All sociodemographic data were without significant difference except for insanitary water supply.

23.8% children with bacterial agents had fever. slightly more than in parasites (21.4%) or in both agents (21.4%), 14.3% IN parasites/*Rota virus*. Vomiting was higher among parasites (30.3%) than bacteria/parasites

and *Rota virus* (18.2%) for each, 15.2% with parasites / bacteria.

Dehydration was common among parasitic 36.0% then with *Rota virus* 16.0% infections. Watery diarrhea was common among parasitic (25.7%) than bacterial or with parasites */Rota virus* (20.0% for each).

Mild diarrhea was common among those without agents (31.8%) or with parasites (30.3%), bacteria (24.2%), moderate diarrhea was common in parasitic (45.8%), (22.9%) in bacterial infections, severe diarrhea was more with *Rota virus* only or with parasitic/*Rota virus* as 22.6% for each, 19.4% with parasitic infection. Visible fecal blood was common among parasitic (32.0%), bacteria (28.0%) or in both 24.0%, fecal pus/mucous were higher among parasitic (35.9%) than bacterial (20.5%) or in both (15.4%). Hepatomegaly mostly detected among parasitic (87.5%), then with both bacteria/parasites (12.5%), all previous differences were significant except for hepatomegaly.

Studied groups		Age g	Test of sizesifiers			
Characteristics	Children ≤ 5 years No.69		Children >5 years No.111		Lest of significance & P value	
Fever ≥38.5					2	
- Yes	17	24.6	25	22.5	$X^2 = 0.1 \& p = 0.7$	
Vomiting						
- Yes	14	20.3	19	17.1	$X^2 = 0.3 \& p = 0.6$	
Dehydration						
- Yes	10	14.5	15	13.5	$X^2 = 0.03 \& p = 0.9$	
Watery diarrhea						
- Yes	12	17.4	23	20.7	$X^2 = 0.3 \& p = 0.6$	
Visible fecal blood						
- Yes	11	15.9	14	12.6	$X^2 = 0.4 \& p = 0.5$	
Fecal Pus \ mucus						
Yes	15	21.7	24	21.6	$X^2 = 0.8 \& p = 0.9$	
Hepatomegally						
	0	0.0	<mark>8</mark>	<mark>7.2</mark>	$X^2 = 5.2 \& p = 0.023*$	
Rota virus						
-Yes	9	13.0	11	9.9	$X^2 = 0.4 \& p = 0.5$	
Presence of bacteria	<mark>18</mark>	<mark>26.1</mark>	<mark>44</mark>	<mark>39.6</mark>	$X^2 = 5.5 \& p = 0.024*$	
Presence of parasites	<mark>29</mark>	42.0	<mark>52</mark>	<mark>46.8</mark>	$X^2 = 10.2 \& p = 0.012^*$	

Table 4: Comparison between children below 5 years and those above according to clinical profile and investigations

Fever, vomiting, dehydration, were slightly more among children < 5 years old than those > 5 years except watery diarrhea, children <5 years had visible fecal blood (15.9%) and Pus/mucus (21.7%) compared to (12.6% & 21.6%) respectively among those > 5years, without statistical significant difference.

Liver affected only in children > 5 years (7.2%), also more bacteria (39.6%) and parasites (46.8%) were detected among them than those < 5 years (26.1%& 42.0% respectively) with statistical significant difference (table 4).

DISCUSSION

Knowledge of the causative enteric pathogens is essential for implementation of appropriate public health measures to control dysentery, in the current study we used different diagnostic laboratory techniques, at least one pathogen was isolated from each stool samples (77.8%), no pathogens were isolated from 40 stool samples (22.2%), intestinal parasites (IPs) were the most frequent pathogens (45,5%), most frequent parasite was G. lamblia (22.2%) followed by E.histolytica (21.0%), Cryptosporidium parvum (9.9%), E.vermicularis (8.6%), *A.lumbricoides* (4.9%), S.mansoni (4.9%), Hookworm (3.7%), H.nana (3.7%), H.hetrophyes (2.4%), Fasciola (1.1%), cases with more than one parasites were 29.7 %, this came in agreement with results obtained by Zabolineja et al, who found that G. lamblia was the most frequent

pathogens causing diarrhea in children¹⁴, in contrast to the findings by Samie et al, who detected that prevalence of *E. histolytica* was higher than *G. lamblia*¹⁵. The 2nd frequent pathogens in our study were bacteria (34.4%).

Our results confirmed previous findings by Thapa et al, who reported that intestinal parasites have been always an important public health problem and it was 16 % in the children with diarrhea as 86 % *G. lamblia*, 32% *E. histolytic*¹⁶, in contrast, Saeed et al, stated that prevalence of bacteria was significantly higher than parasitic and viral enteric pathogens, explaining the low positive intestinal parasites to availability of safe sources of drinking water and the location of public toilets at a distance from houses¹⁷.

In our study, Bacteria was the 2nd frequent causative agents (34.4%) of this, diarrhea- genic E. coli was the most frequent pathogen (42.0%) followed by Salmonella spp. (25.8%), Shigella spp. (17.7%) and Klebsiella (14.5%), this came in agreement with results obtained by Saeed et al &Al Mizury et al, who found that E. coli was the most frequent pathogen among children with diarrhea (48%, 7.7%), Salmonella spp. (4%, 3.5%), Shigella spp. (8%, 2%) respectively^{17,18}. The 3rd prevalence was *Rota virus* (11.1 %), in contrast to our results, Youssef et al, reported that Rota virus was the most prevalent causative agent in 33% of hospitalized children with diarrhea¹⁹. Previous results confirmed previous findings by Thapa et al, who found that intestinal parasites (IPs) have been always an important public health problem and the rate of IPs was 16 % in the children with diarrhea as 86 % G. lamblia,

32% *E. histolytic*¹⁶, in contrast, Saeed et al, reported that the prevalence of bacteria was significantly higher than parasitic and viral pathogens ¹⁷. In our study, cases with single parasite (35.6%) were higher than cases with more than one parasite (27.9%), single bacterial cases were (21.7%) and (9.4%) in cases with parasitic/ bacterial and (4.4%) in cases with parasitic / *Rota virus* infections.

Our results detected that highest rate of pathogens among children ≤ 5 years were *parasites* (42.0%) followed by *bacterial* (26.1%) and *Rota virus* (13.0%), while in children > 5 years it was 46.8% in parasitic, 39.6% in bacterial and 9.9% in *Rota virus* which is a significant result Ansari et al reported that prevalence of pathogens was higher in children < 2 years of age²⁰, in contrast to Youssef et al, who stated that children < 5 years of age were more likely to have dysentery than in younger¹⁹.

Our results detected that prevalence of dysentery in children ≤ 2 years with history of breast feeding was lower than in those with no breast feeding history (46.7%, 53.3%) respectively, this came in agreement with result obtained by Lamberti et al, who stated that the significance of breast feeding in protection against infectious agents during infancy as well preventing hospitalization for children with diarrhea, decreasing prevalence of morbidity and mortality ²¹.

Our results stated that the majority of watery diarrhea was 20.7% in children >5 years, it was 17.4% in children 5 \geq years, while it was 17.4% in children 5 \geq years. Liver play an important role in host immune system defense, liver can vary affected with wide variety of manifestations from a- symptomatic elevations in amino-transaminase, liver failure, fibrosis or cirrhosis 23 , this is in agreement with our results as hepatomegaly was 7.2% in children >5 years, mostly in parasitic infection (87.5%) with no significant difference. The most frequent pathogens in boys were the intestinal parasites (40.9%), followed by bacterial (14.8%), while it was (30.4%, 28.3%) in girls respectively. Rota virus was high in boys (5.7%) than in girls (2.2%), this came in agreement with results which reported that rate of pathogens in boys with diarrhea was higher than in girls ¹⁶.

In our study, all children presented with diarrhea, the rate was more higher in girls (51.1%) than in boys (48.9%) with no significant differences, in contrast, Saeed et al, detected a higher rate of diarrhea in boys (55%) than in girls $(45\%)^{17}$. The ratio of boys to girls with diarrheal diseases was distinct from that in other study by Mashoto et al, who reported that male and female children were equally affected ²². Visible fecal blood was higher in parasitic (30.2%) than in bacterial infections (28.0%), these come in agreement with results obtained by Saeed et al, who found that visible fecal blood was more in parasitic infections especially in *E. histolytica* (20%)¹⁷. Also, rate of fecal pus/mucous

were more in parasitic (35.9%) than in bacterial infections (20.5%).Watery diarrhea was more common than bloody diarrhea (19.4%, 13.9% respectively). We detected that common associated signs and symptoms with diarrhea(100 %) were fever (23.3%) vomiting (18.3%) and dehydration (13.9%), fever was slightly higher in bacterial (23.8%) than in parasitic (21.4%) or with Rota virus infections (14.3%) with significant statistical difference .vomiting was more common in parasitic infections (30.3%) than those with bacterial (18.2%), or Rota virus infection (9.1%) respectively with significant statistical difference, dehydration was more in parasitic (36.0%) than in viral (16.0%) or bacterial infections (8.0%) with significant statistical difference, this come in agreement with results obtained by Saeed et al, who also detected fever in 57.9%, vomiting in 56.8% ,dehydration in16.5% among children with diarrhea 17

We detected that mild diarrhea was common among children with no agents (31.8%) while it was 30.3% in parasitic, 24.2% in bacterial infections, moderate diarrhea was more common in parasitic (45.8%) than bacterial infection (22.9%), while severe diarrhea was more among those infected by *Rota virus* only(22.6%) equally to those with parasitic infections, the rate was more frequent in parasitic (25.7%) and 20.0% in bacterial or in Rota virus / parasitic infections .visible fecal blood was higher in parasitic (30.2%) than in bacterial infections (28.0%),these came in agreement with results obtained by Saeed et al which reported that visible fecal blood was more in parasitic infections specially in *E. histolytica* (20%)¹⁷.

We detected that fecal pus /mucous were more in parasitic (35.9%) than in bacterial infections (20.5%) was more common than bloody ,watery diarrhea diarrhea (19.4%, 13.9% respectively), and the rate of parasitic infections among those with insanitary water supply were 50.0% which is higher than in bacterial (17.9%) or parasitic/bacterial infections (10.7%), as well as in children with insanitary sewage disposal systems, 48.1% for parasitic, 11.1% for bacterial & 11.1% in mixed infections respectively with no significant statistical differences. Al Mizury et al, reported that children of low housing level at higher risk (3%) than those with good housing level (1.4%)indicating that availability of house hold sanitation facilitates access to clean water, good personal hygiene and better nutrition¹⁸. Children with illiterate parents had 28.9% parasitic, 26.3% bacterial, 2.6% Rota virus, and 10.5% in both mixed parasitic / bacterial infections, among those with mild educated parents, 44.9% had parasitic, 19.2% had bacterial, 3.8% had Rota virus and 10.3% had parasitic / bacterial infections, while among those with moderate education, parasitic infections were 25.9%, 24.1% bacterial and 9.3% in mixed infections, among those with well- educated parents 40% had no enteric pathogens and 40% had parasites, 10.0%

bacterial and 10.0% mixed parasitic / bacterial / Rota virus infections significant statistical difference.

CONCLUSION & RECOMMENDATION

This study highlights the frequency of dysentery in children and the importance of parasites, bacteria and Rota virus as causative agents, intestinal parasites were the most common agents followed by bacterial pathogens, awareness about prevention, improved hygiene and proper medications are needed to reduce the disease, more information about source, mode of transmission and risk factors are needed to develop recent controlling methods strategies.

Conflicts of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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