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Study of Intestinal Parasites and Their Effects on Some Blood Parameters Among Children Suffering from Diarrhea in Zakho district - Iraq.

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ABSTRACT

Background and Objective: The intestinal parasite infection is one of the major health problems in developing countries, particularly in tropical and subtropical areas in children. Hence, this cross-sectional study investigated the prevalence of intestinal parasitic infections and effects on some blood parameters among diarrheic children from October 2024 to May 2025 in Zakho district, Kurdistan Region, Iraq. Methodology: Stool samples were collected from 403 diarrhea children among the ages (<1-15 years) in Hospital and camp in Zakho city. The stool was examined microscopic by direct wet mount and modified Ziehl-Neelsen stain and also for hematological study. Sixty samples from infected children and 30 samples from non-infected children, the blood was examined by a blood analyzer. **Results:** The overall prevalence of intestinal parasite infection was 33.25%. Three species of intestinal parasites recorder: Entamoeba spp. (63.43%), Cryptosporidium spp. (44.77%), and Giardia lamblia (5.22%). The percentage of infection in females was higher than that of males (34.1% vs 32.32%). Also found the high infection in age group 2-5 years, rural residents, and unemployed parents at rates of 36.1%, 37.7%, and 35.1%, respectively. Monthly peaks infection occurred in April and October. The level of hematological parameters (Hb, PCV, and MCV) decreases in the infected children, while the level of WBC and vitamin B12 increases in infected children. Conclusion: These findings highlight a high IPI burden in Zakho, the critical role of environmental, socioeconomic, and hygiene factors. Comprehensive diagnostics and targeted health interventions are crucial to address these parasitic infections and improve child health in the region.

INTRODUCTION

Intestinal parasitic infections (IPIs) represent a significant global public health challenge, particularly in developing countries, where factors such as inadequate sanitation, poor hygiene practices, and limited access to safe drinking water contribute to their widespread prevalence (Al-Maqtari *et al.*, 2024). Children are disproportionately affected by these infections due to their underdeveloped immune systems and increased exposure risks, leading to a range of adverse health outcomes including malnutrition, growth retardation, and various gastrointestinal disorders, most notably diarrhea (Bayoumy *et al.*, 2018a; Al-Musawi *et al.*, 2023). Most of the intestinal parasites are transmitted to the host by ingestion of contaminated foods and drinks or by getting in contact with contaminated soil where infection occurs through skin penetration by the infective larva stage (Ahmed *et al.*, 2022).

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The main intestinal parasites affecting humans include *Entamoeba histolytica*, *Giardia lamblia*, *Hymenolepis nana*, *Cryptosporidium* spp., *Taenia saginata*, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Trichuris trichur* and Hookworms (Hamad, 2023).

In addition to the gastrointestinal symptoms, intestinal parasitic infections profoundly impact physiological systems, including hematological parameters, can lead to significant blood loss, malabsorption of essential nutrients like iron and vitamin B12, and compromised nutritional status, often culminating in anemia (Bayoumy et al., 2018b; Al-Saad & Abdul-Sahib, 2021a; Hemdad, 2023). Several studies have investigated these hematological alterations, showing correlations between parasitic infections and changes hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), corpuscular hemoglobin (MCH), white blood cell (WBC) counts, and serum ferritin levels (Bayoumy et al., 2018a; Al-Masoudi et al., 2020a; Al-Saad and Abdul-Sahib, 2021a; Al-Sudani et al., 2024). For example, research indicates that infected individuals may exhibit lower Hb and PCV values and altered WBC counts compared to non-infected individuals, with some parasites like Ascaris lumbricoides being particularly associated with macrocytic RBCs (Al-Saad & Abdul-Sahib, 2021a).

In Iraq, IPIs remain endemic across various regions, affecting diverse population groups including children, and are often associated with significant morbidity (Al-Mehemdi et al., 2020). Studies conducted in Kurdistan Region and Iraq have consistently reported varying, yet notable, prevalence rates of intestinal parasites among diarrheic patients and school-aged children, for instance, in Zakho City (Mero et al., 2015); in Wasit province (Al-Saad & Abdul-Sahib, 2021b); in Baghdad (Al-Musawi et al., 2023a); in Bagubah (Al-Magtari et al., 2024) and in

Duhok province (Al-Sulaiman & Al-Jubouri, 2024). The profound impact of these infections on children's health, particularly their nutritional and hematological status, underscores the critical need for continued research and effective intervention strategies, this study aims to investigate the prevalence of intestinal parasites and their associated impact on specific blood parameters among children suffering from diarrhea in Zakho district, Kurdistan Region, Iraq. examining these crucial aspects, research seeks to contribute to a deeper understanding of the local epidemiological landscape and provide evidence that can inform targeted public health interventions to improve child health outcomes in the region.

MATERIALS AND METHODS Study Subjects and Sample Collection:

This cross-sectional study included a total of 403 stool samples collected from children suffering from diarrhea in different ages (<1 to 15 years) from Zakho Emergency Hospital, General Zakho Hospital, Chamishko Camp and Bersive Camp in Zakho district from late October 2024 to May 2025. For each participating child, take some information according to the questionnaire including gender, ages, and other information and then utilize data analysis.

Laboratory Diagnosis:

Stool Examination: Stool examination primarily examined macroscopic and microscopic identification of parasite stages using various techniques.

Macroscopic Examination: The stool samples examined primarily grossly, which included consistency, color, presence of blood or mucus, and some live worms (Calderaro *et al.*, 2006).

Microscopic examination:

1. Direct Wet Mount Method:

The direct wet mount is a fundamental and rapid microscopic technique for the initial examination of fresh stool samples. A small portion of stool

is emulsified in saline and/or Lugol's iodine on a slide, allowing for the detection and preliminary identification of various parasite forms, including motile trophozoites, cysts, eggs, and larvae. Examination begins under 40X magnification to scan the field, followed by 100X magnification for detailed identification and differentiation of smaller parasite structures based their morphology and any observed motility.

2. Modified Ziehl-Neelsen Stain for Cryptosporidium Parvum:

The Modified Ziehl-Neelsen (MZN) staining technique is a crucial acidfast method specifically utilized for the detection of Cryptosporidium parvum oocysts, which are often too small and indistinct for reliable identification with routine wet mounts. After preparing and stool smear put in methanol for fixation for 10 minutes, it is stained with carbol fuchsin. decolorized with acid-alcohol, and then counterstained with methylene blue or malachite green. Under 100X oil immersion microscopy, Cryptosporidium parvum oocysts are observed as small, brightly red or pink, acid-fast bodies against a contrasting background, owing to the unique composition of their oocyst walls that resist decolorization.

Hematological & Immunological Study:

For this purpose, 5 ml of blood samples were withdrawn of 60 children with intestinal parasites (28 males and 32 females), in addition to 30 blood samples from children without intestinal parasites (14 males and 16 females) with a sterile 5 ml disposable syringe. Put 3 ml of blood in a gel plastic tube without anticoagulation,

then placed in the centrifuge at 5000 rpm for 10 minutes. The serum is placed in a small tube of Eppendorf tubes and stored at -20 °C for later use for immunological examination. Also, 2 ml of blood drawn in a special tube containing the EDTA (Ethylenediaminetetraacetic Acid) for the hematological parameters using the Sysmex (XN-350) dives.

Statistical Analysis:

All collected data from questionnaires and laboratory analyses will be entered into a suitable statistical software package (SPSS Statistics) using Chi-square tests and t-tests. A *p*-value of less than 0.05 will be considered statistically significant for all analyses.

RESULTS AND DISCUSSION

The current study, conducted in Zakho district, the results are illustrated in Table (1), the overall rate of intestinal parasitic infections (IPIs) among children with diarrhea was 33.25%. This rate is notably higher than the other studies recently conducted in Kurdistan region and other parts Iraq including in Karbala was 13% (Al-Sultany and Al-Morshidy, 2023), in Baghdad was 27.3% (Saleh, 2023), and also in Duhok city each reported rate was 27.7% and 21.27% (Ismael et al., 2024; Hassan and Meerkhan, 2025), On the other hand, is agree with study in Erbil and Duhok (Hama and Rahemo, 2014; Hassan & Mero, 2020) reported rate 32.2% and 28.3%, respectively. The variation in these rates might be attributed to the type of population involved in these studies or poorer living conditions in displacement camps or to the number of specimens tested (Mero and Hussein, 2013).

Table 1: The prevalence of parasites in the examined diarrheic stool specimens.

No. examination	No. of infection	Prevalence %
403	134	33.25%

As a result, in Table (2), regarding the gender, the infection rate in females was higher in comparison to males (34.1% vs 32.3%), respectively. This study found non-significant difference in infection rates

between males and females (P = 0.698). Similarly, studies performed in Duhok and Baghdad (Salih, 2022; Saleh, 2023) in which higher infection rates were reported among females than males which were

55.6% vs 44.4% and 67.3% vs 32.67%, respectively. While the current results disagree with previous findings in Karbala and Duhok, were reported the higher infection rates in males than females (56.66% vs 43.33% and 22.03% vs 20.45%), respectively (Al-Sultany and Al-Morshidy, 2023; Hassan & Meerkhan, 2025). The higher rates in females may be due behavioral of females, immunological, and hormonal variations may be the cause of the difference in infection rates between males and females (Sellau *et al.*, 2020).

In terms of age, in Table (2), showed the highest rate of infection was reported among aged 2-5 years was 36.1%, followed in the 10-14 years was 35.8%. Statistical analysis is not significant between the ages (P = 0.519). The present results agree with results of (Al-Hasheme et al., 2020) in Kerbala, who found infection rates of 20.34% in children of ages closer to the ages selected in this study (group 1 to 5 years). While contradict with the Al-Sultany and Al-Morshidy (2023) in Karbala city and Ismael et al. (2024) in Duhok city, were both of them reported the highest infection rate (57.1% and 17.2%) among children aged 5-10 years. The increase in the rate of infection may be attributed to different factors, children are more active and playing outdoors, or due to the children have decreased immune systems or to the application of personal hygiene enable them to acquire infectious agents.

Regarding the number of family members showed a positive correlation with infection rate in Zakho city, the children from families of 7 to 9 members being more infected (39.8%) than those from (6 and less) and (10 and above) families reported lower infected (29.6% and 35.6%). Statistically, the differences were not significant (P = 0.145). These results were agreement with findings from Baghdad and Duhok, they showed the highest rate of infection (64.8% and 74.17%) among families with more than 5 members and the lowest rate (31.1% and 25.83%) among families with less than 5 members (Salman

et al., 2019; Salih et al., 2022). On the other hand, the current results disagree with the study performed in Baghdad/Iraq (Saleh, 2023) that showed the highest rate of infection in families less than 5 members (86%) and lowest rate in families more than 5 members (14%). Where larger families were shown to experience greater infection due to overcrowding and shared sanitation facilities (Salman et al., 2019; Salih et al., 2022).

The analysis of intestinal parasite distribution by lab location revealed a slightly higher infection rate in camps (36.1%) compared to hospitals lab (29.9%), though this difference was not statistically significant (P = 0.146). This observation could be attributed to variations in living conditions, sanitation, and camps, often characterized by higher population density, can facilitate easier transmission parasitic infections. According to residency, the prevalence of intestinal parasites among children living in rural areas was significantly (P = 0.029) higher as compared with urban residents (37.7%) and 27.3%), respectively. Similarly, the present results agree with studies conducted in Egypt (Hussein et al., 2021) and in Duhok city/Iraq (Salih et al., 2022) each of the reported higher infection rates in residents of rural area than (37.9% and 59.58%), respectively. Contrastingly, a study in Baghdad reported higher urban infection rates than rural (98% vs 8%), possibly to overcrowded urban due settlements with poor sanitation (Saleh, 2023). These findings suggest that limited access to safe drinking water, contamination from agricultural practices, and often less consistent hygiene, all of which create a more conducive environment for parasitic transmission (Mero and Hussein, 2013).

Regarding to the type of drinking water, a significant association was found between the type of drinking water and infection rates (P = 0.023), with children consuming chlorinated and unclear water sources showing higher infection rates

(39.3% and 37.2%) than those using well water (24.3%). The observed results are agreed with a study performed in Baghdad and Duhok, where improper treatment and post-source contamination contributed to increased parasitic infections (Salih et al., 2022; Saleh, 2023). This indicates that chlorination alone may not be sufficient to eliminate all parasitic risks, particularly when water quality infrastructure is compromised. According to socioeconomic status (SES) emerged as a critical determinant in the current study. Children from families categorized as not well had a highly significant (P = 0.00)infection rate (43.3%) compared to those from moderate and well-off families (23.1% and 21.7%), respectively. The observed results are in line with a study performed in Iraq, including in studies from Diwaniyah and Duhok, which linked poor SES to limited access to hygiene resources, poor nutrition, and lower awareness of disease prevention strategies (Al-Waaly et al., 2020; Salih et al., 2022).

Washing the fruit and vegetables was another risk factor, the children consuming improperly washed food having higher infection rate (40.4%) than the well (21.6%),children washed statistically these differences were highly significant (P = 0.00). This finding is consistent with the Duhok study, which reported 24.73% infection among children consuming unwashed fruits and vegetables (Hassan & Meerkhan, 2025). It underscores role of contaminated food transmission, particularly in environments where access to clean water for food preparation is limited. Furthermore, poverty, low socioeconomic, lack of health education programs, poor sanitation and hygiene application are all linked to these variables. Regarding employment, in Table

2 showed the unemployed parents of child was higher infection rate (35.1%) than the employed parents of child (29.6%), also the statistical non-significance between each of them (P = 0.273). According to the study (Alum *et al.*, 2010) who emphasize the strong association between lower socioeconomic status and higher prevalence of parasitic infections globally.

The monthly analysis revealed fluctuating in the infection of intestinal parasites among diarrheic children in Zakho city, was observed in Table (2), the highest rates of infection recorded during April 2025 and October 2024 (38.5% and 36.4%), respectively. While the lowest rates of infection were 20% in January 2025, although the difference between the number of cases and monthly distribution of parasites was statistically not significant (P = 0.757). This monthly pattern is consistent with other studies in the region, such as the study in Zakho city (Al-Bajalan and Al-Khayat's, 2022), who also reported higher infection rate during April was (37%) linked to spring rainfall facilitating parasite transmission. Also, in Duhok (Hassan et al., 2021) reported the lower rates of infection during January (20%), while the higher rates of infection were found in February (35%), potentially reflecting year-to-year variations in temperature or hygiene campaigns. This variation on months in the rate of infection may be due to the presence of reservoir hosts to the abundance of insects that act as mechanical vectors, to the sources of water used for irrigation of vegetables high temperatures, insufficient rain or moisture, and climatic conditions that differ by latitude and longitude all these factors help in the variations of the prevalence of parasite diseases throughout the year (Al-Garawi, 2015).

Table 2: Prevalence of	parasite among the	population in relation	to some variables (N=403).

Variable		No.		Infection		nfection	Ì
		Examined	No.	%	No.	%	Significant
G 1	Male	198	64	32.32%	134	67.7%	$X^2 = 0.151$
Gender	Female	205	70	34.1%	135	65.9%	P = 0.698
	1M - 1Y	28	7	25.0%	21	75%	
A C	2Y - 5Y	108	39	36.1%	69	63.9%	$X^2 = 2.267$
Age Groups	6Y – 9Y	130	39	30.0%	91	70.0%	P = 0.519
	10Y – 14Y	137	49	35.8%	88	64.2%	
Lab	Hospitals Lab	187	56	29.9%	131	70.1%	$X^2 = 6.820$
Location	Camps	216	78	36.1%	138	63.9%	P = 0.146
Family	6 and less	240	71	29.6%	169	70.4%	$X^2 = 3.864$
Members	7 to 9	118	47	39.8%	71	60.2%	$A^{2} = 3.804$ P = 0.145
Wiellibers	10 and above	45	16	35.6%	29	64.4%	I = 0.143
Residence	Urban	172	47	27.3%	125	72.7%	$X^2 = 4.746$
Residence	Rural	231	87	37.7%	144	66.7%	P = 0.029
Duinling	Well water	136	33	24.3%	103	75.7%	X2 = 7.585
Drinking water	Chlorinated	84	33	39.3%	51	60.7%	A2 = 7.383 P = 0.023
water	Not clear	183	68	37.2%	115	62.8%	I = 0.023
~ ·	Well	175	38	21.7%	137	78.3%	
Socioecono	Moderate	13	3	23.1%	10	76.9%	X2 = 20.797
mic status	Not well	215	93	43.3%	122	56.7%	P = 0.00
Washing	Well	148	32	21.6%	116	78.4%	$X^2 = 14.252$
fruit and vegetable	Moderate	255	102	40.0%	153	60.0%	P = 0.00
Employme	Employed	135	40	29.6%	95	70.4%	$X^2 = 1.199$
nt	Unemployed	268	94	35.1%	174	64.9%	P = 0.273
	Oct 2024	44	16	36.4%	28	63.6%	
	Nov 2024	12	3	25.0%	9	75.0%	
	Dec 2024	18	6	33.3%	12	66.7%	
Months	Jan 2025	20	4	20.0%	16	80.0%	$X^2 = 5.008$
INTOHUMS	Feb 2025	29	10	34.5%	19	65.5%	P = 0.757
	Mar 2025	133	40	30.1%	93	69.9%	
	Apr 2025	122	47	38.5%	75	61.5%	
	May 2025	25	8	32.0%	17	68,0%	

In Table (3), only recorded protozoan parasites, with the high rate of infections were *Entamoeba* spp., at 63.43%, followed closely by *Cryptosporidium* spp. at 44.77%, *Giardia lamblia* and *Entamoeba coli* accounted for a smaller proportion of 5.22% and 1.49%, respectively. This dominance of *Entamoeba* spp. and *Cryptosporidium* spp. is consistent with other studies observed in another parts of Iraq, for instance, in Erbil (Al-Daoody *et al.*, 2021) stated that *E. histolytica* was the most prevalence parasite (80.1%), followed by *G. lamblia* (19.8%) and *H. nana* (0.1%).

Also, in Duhok city (Salih et al., 2022) were reported the highest rate (21.67%) with E. histolytica, followed by G. lamblia (5.46%), *B. hominis* (0.34%). Another study in Karbala (Al-Sultany and Al-Morshidy, 2023) reported the highest rate (10.54%) with E. histolytica, followed by G. lamblia (2.46%), and *Cryptosporidium* spp. (0.4%). In Duhok province by Al-Saeed et al. (2024) similarly reported E. histolytica (78.0%) and *C. parvum* (17.1%) as leading protozoan infections among children with diarrhea. On the other hand, the current result disagrees with study in Duhok by Mero and Hussein (2013) found *G. lamblia* (11.92%) and *E. histolytica* (10.15%) to be the most common. According to the (Hadi, 2011) the high prevalence of amoebiasis is attributed to their direct life cycle and

transmission in addition to the contaminated water, poor preventive measures and health education all these factors contribute in the prevalence of these parasites.

Table 3: Distribution of intestinal parasitic infection percentages in children according to species of the parasite (total number of infections = 134)

Parasite species	Infection number	Percentage (%)
Entamoeba spp.	85	63.43%
Cryptosporidium spp.	60	44.77%
Giardia lamblia	7	5.22%
Entamoeba coli	2	1.49%

The results in the Table (4) showed the single and double infection of parasites, it reveals that single infections are predominant the infection rate was 85.07% (114/134), with Entamoeba spp. was (50.74%), Followed by Cryptosporidium spp. (26.62%), Giardia lamblia and Entamoeba coli for each of them (2.98% and 1.49%), respectively. The number of double infections was less frequent. constituting 14.92% (20/134). In all of these cases the high co-infection between Entamoeba spp. and Cryptosporidium spp. was 12.68% (17/134), followed by Giardia lamblia and Cryptosporidium spp. the rate of infection was 2.23% (3/134). This pattern of co-infection underscores the

complex interplay of parasitic agents in diarrheal disease in the region. Similarly, Al-Taei (2019) in Babylon province, recorded single infection at a rate of 99.51% was higher than double infections at a rate (0.49%). Also, in Erbil (Al-Daoody et al., 2021), the single infection recorded at a higher rate (99.61%) than mixed infection was recorded at a rate (0.36%). The high rate of *Entamoeba* spp. and Cryptosporidium spp. in your data aligns with their high individual prevalence and common environmental suggests risk factors. emphasizing the need comprehensive diagnostic and public health interventions to address the concurrent burden of these parasites in children.

Table 4: The prevalence of various species of intestinal parasites (single and double) according to type of parasite

Pa	rasites	No. of Infected	Percentage %
	Entamoeba spp.	68	50.74
Single infections	Cryptosporidium spp.	40	29.85
Single infections	Giardia lamblia	4	2.98
	Entamoeba coli	2	1.49
Su	btotal	114	85.07
Double infections	Entamoeba spp. + Cryptosporidium spp.	17	12.68
	Giardia lamblia + Cryptosporidium spp.	3	2.23
Su	ıbtotal	20	14.92
Total		134	100

Table (5) shows the means of hematological parameters in children infected with intestinal parasites and control children in the Zakho city. The results of this study showed highly significant (P =0.00) in the WBCs, Hb, PCV and MCV in children infected with intestinal parasites compared with non-infected children, while non-significant (P = 0.230) in the vitamin B12 in children infected with intestinal compared with non-infected parasites children. The reduction in hematological parameters (Hb, PCV and MCV) is due to intestinal parasites that colonize the digestive system, especially intestinal cause bad absorption of sugars, fats, vitamins, folic acid, iron, and zinc, which causes anemia because it enters the composition of hemoglobin (Dos Santos et al., 2013; Ehiaghe et al., 2013). Similar, the study conducted in Basrah and Baquba city (Alsamir and Alabdullah, 2020; Awaad et al., 2022) each of them reported decrease the rate of Hb, PCV and MCV in the infection children with parasites. It means when E. histolytica is present in the intestines, it can cause gastrointestinal

problems because the trophozoite attaches to the intestinal villi, absorbs nutrients, secretes proteins that analyze the host's tissues and cells, and feeds on red blood cells, which can cause the necrosis of the intestinal mucosa (Ghimire & Mishra, 2005; Adday, 2009). The parasite Giardia damages the mucosa of the gastrointestinal tract, leading to malabsorption syndrome, particularly with regard to vitamins (Vitamin B12) and iron (Júnior et al., 2016; Olivares et al., 2002). The increasing number of white blood cells may be due to their role in the response of the immune system to the treatment and elimination of intestinal parasites (Ustun et al., 2004), it consistent with previous research linking parasitosis to leukocytosis (Al-Megrin et al., 2020; Awaad et al., 2022). While the level of vita B12 is fluctuating among the infection children with intestinal parasites for this reason the level of Vita B12 recorded in the study more than the noninfection children. These results underscore the need for integrated parasitic screening and hematological monitoring in pediatric diarrheal cases in endemic regions.

Table 5: Comparative between hematological parameters in infected and Control children with intestinal parasites.

Hematological parameter	No. examined	M±SD	P value	
WBC	Infection: 60	9.30±2.19	P = 0.00	
WBC	Control: 30	6.93±0.92	P = 0.00	
HGB	Infection: 60	9.47±0.58	P = 0.00	
пов	Control: 30	13.06±0.86	P = 0.00	
DCV	Infection: 60	29.85±1.14	D 0.00	
PCV	Control: 30	39.99±1.63	P = 0.00	
MCV	Infection: 60	72.03±2.30	P = 0.00	
IVIC V	Control: 30	84.94±4.54	F = 0.00	
Vita B12	Infection: 60	513.55±328.42	P = 0.230	
vita D12	Control: 30	440.49±45.06	F = 0.230	

Conclusions

This study reveals the infections of intestinal parasitic among children in Zakho district was high. Our findings underscore that environmental and socioeconomic factors are critical

determinants of these infections. Specifically, children living in rural areas, those consuming chlorinated or unclear water sources, individuals from "not well" socioeconomic backgrounds, and those who consume improperly washed fruits and

vegetables face a significantly higher risk of infection. This research contributes vital local epidemiological data, emphasizing the urgent need for comprehensive diagnostic strategies and targeted public health interventions. Such interventions should focus on improving water sanitation, promoting hygiene education, and addressing socioeconomic disparities to effectively mitigate the impact of IPIs and enhance child health outcomes in the region.

Declarations:

Approval: The Ethical study was with performed in accordance the Declaration of Helsinki - Ethical Principles for Medical Research, revised in 2008, and was approved by the Ethics Committee of Zakho Directorates of Health (NOV2024/UOZE24 ON 12/11/2024) for examining students taking samples, consent and Data.

Competing interests: There is not any conflict of interest in this study.

Availability of Data and Materials: The data used in this study are available on request from the corresponding author.

Authors Contributions: Both authors made substantial contributions to this article. B. M. Khorsheed and A.R. Issa conceived and designed the work. B. M. Khorsheed collected the samples and data, performed the analysis and wrote the first draft of the manuscript. A.R. Issa revised the data analyzed and reviewed the manuscript. Both authors have read and agreed to the published version of this manuscript.

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