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EPIDEMIOLOGY, SEASONAL VARIATIONS AND RISK FACTORS OF PEDIATRIC GASTROENTERITIS IN DISTRICT MARDAN

Kainat[1], Ruqiya Pervaiz*[1], Gauhar Rehman[1], Fawad Khan[2]

[1]Department of Zoology, Faculty of Chemical and Life Sciences, Abdul Wali Khan University, Mardan, KP, Pakistan, Email: kainatkhan81082@gmail.com , ruqiyapervaiz@gmail.com , gauhar@awkum.edu.pk
[2]Department of Biotechnology, Faculty of Science, Comsats University Islamabad, Abbottabad campus, Email:fawad9468@gmail.com

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Corresponding Author:
Ruqiya Pervaiz,
Department of Zoology, Faculty of Chemical and Life Sciences, Abdul Wali Khan University, Mardan, KP, Pakistan
ruqiyapervaiz@gmail.com

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ABSTRACT

Gastroenteritis remains a leading cause of morbidity and mortality in children, particularly in developing countries. This study aimed to investigate the prevalence, associated risk factors, clinical features, and bacterial causes of gastroenteritis in children from District Mardan, Khyber Pakhtunkhwa, Pakistan. A cross-sectional study was conducted on 250 pediatric patients presenting with symptoms of gastroenteritis. Clinical data were collected through structured questionnaires and physical examination. Stool samples were analyzed macroscopically and microscopically, followed by bacterial culture on selective media. Molecular identification of *Salmonella spp.*, *Shigella spp.*, and *EPEC* was performed using Polymerase Chain Reaction (PCR) targeting invA, ipaH, and eaeA genes, respectively. Diarrhea (100%), vomiting (72%), and fever (68%) were the most common clinical symptoms. Bacterial pathogens were isolated in 52 (*Salmonella spp.*), 33 (*Shigella spp.*), and 47 (*EPEC*) cases, with PCR confirmation rates of 94.2%, 93.9%, and 95.7% respectively. The highest prevalence occurred during summer (38.4%), and significant risk factors included lack of handwashing, untreated water use, poor sanitation, and low parental education. Form the statistical analysis it is confirmed that relations among these variables and infection risk ($p < 0.05$). The result shows a high intensity of gastroenteritis among children in Mardan District, strongly influenced by environmental and socioeconomic factors.

1. INTRODUCTION

Gastroenteritis, an inflammation of stomach and intestines, mainly an acute or chronic disease, is typified by diarrhea, vomiting, nausea, cramps of the stomach, fever and the dehydration of the body. It is one of the most established causes of morbidity and hospitalization in children worldwide especially in the low and middle-income countries (LMICs) like Pakistan (Guerrant et al., 2013). Among children below the age of ten, gastroenteritis causes morbidity and mortality because of their underdeveloped immune systems and the susceptibility to dehydration and under nourishment (Walker et al., 2013). Infectious agents are the main cause of this disease and the most common etiology is the viruses especially rotavirus, norovirus, adenovirus, and astrovirus. There is also a significant involvement of bacterial-like Escherichia coli, Salmonella spp., Shigella spp., Campylobacter jejuni, protozoal organisms, including Giardia lamblia, and Entamoeba histolytica, in the outbreaks of diseases, particularly in regions with impaired water and sanitation grounds (Kotloff et al., 2013).

Gastroenteritis load in Pakistan is very high. It is within the top five factors that lead to death among children less than ten years old, where it is estimated to cause 53,000 deaths a year (UNICEF, 2022). Unhealthy hygiene habits, polluted drinking water, insufficient sanitation system, and ignorance on food safety play a major role in the widespread prevalence of the ailment (Khan et al., 2020). Most residential areas in rural and semi-urban areas, such as District Mardan, source untreated water by the use of wells, hand pumps, and rivers; these sources are prone to contamination by human and animal fecal waste. The lack of effective sewage systems adds even more to the danger of transmitting gastroenteritis-causing pathogens through water (Ashraf et al., 2017).

Malnutrition and under nutrition that is common in most of Khyber Pakhtunkhwa region of which Mardan is part further adds to the effects of the gastroenteritis in children by compromising the immune system by weakening the system and making it hard to recuperate after experiencing repeated infections (Black et al., 2013). This just sets a vicious cycle because the future infections will be susceptible to repeated episodes of diarrhea and poor absorption of nutrients which causes poor growth. The WHO has come out strongly to support the idea of the integrated approach to disease management, and nutrition in order to end the cycle and mitigate death rates related to gastroenteritis (WHO, 2023).

Socioeconomic determinants such as lack of access to education, low-income, health conditions, poor access to medical care are also key factors influencing prevalence of diseases. Illustrative studies have revealed that underprivileged children and those born to mothers who have not received basic health education have augmented risks associated with the contraction of gastroenteritis and related complications, when they are delayed in treatment and preventive measures are not undertaken (Lamberti et al., 2011). Furthermore, the poor feeding strategies of infants, including early discontinuation of exclusive breastfeeding, use of unhygienic water during bottle feeding, and weaning among nursing babies with contaminated food, also subject the children to gastrointestinal infections (Bhandari, et al, 2003).

Although countries, including the introduction of the rotavirus vaccine in 2018 during the Expanded Programme on Immunization (EPI) have conducted national campaigns, rural and underserved areas have not had the overall prevalence of vaccinations as they introduce logistic and vaccine hesitancy problems (GAVI, 2020). Therefore, vaccine-preventable diarrhea continues to be a significant threat to children health. Also, there is a mounting antimicrobial resistance challenge in bacterial gastroenteritis therapy since the random use of antibiotics has given rise to the development of multidrug-resistant strains that make the treatment challenging (Ahmed et al., 2020).

The World Health Organization (WHO) estimates that childhood diarrheal disease caused by gastroenteritis makes up more than 1.7 billion cases per annum claiming a number of nearly 525,000 childhood deaths per annum (WHO, 2023). It is worse in the underdeveloped and developing nations where there is low access to some basic sanitation, good hygiene and clean drinking water. The main difficulties that occur in these countries are unclean water supplies, inadequate waste management, overcrowded living conditions, and the absence of health education, all of which play a major role in the swift transfer of the gastrointestinal pathogens (UNICEF, 2022; Guerrant et al., 2013).

Gastroenteritis is being a recurring social health concern with morbidity and mortality in children less than ten years old and a leading explanation of death and ailment in Pakistan. It is approximated that approximately 50,00053, 000 of the children in Pakistan die every year as a result of the complications of diarrhea, and most of them can be attributed to viral and Bacterial Gastroenteritis (Khan et al., 2020). It is most notable on rural districts such as District Mardan Khyber Pakhtunkhwa, because of the socio-economic problems, lack of clean water resources, poor hygiene, and inaccessible medical care. Lack of effective sewage connection and drinking water filtration system also increase risk and contamination is a major issue, particularly, in the monsoon season, where flow of water on the surface and wells can easily get contaminated (Ashraf et al., 2017).

Although significant progress has been made in the provision of healthcare services and the administration of the Expanded Programme on Immunization (EPI)- the rotation vaccine was introduced in 2018 to address one of the most common triggers of viral gastroenteritis in children- the coverage and awareness especially in rural settings are not ideal (GAVI, 2020). A large percentage of the population in such areas as District Mardan does not have access to inoculation facilities or does not have information on the vaccination plan. Additionally, core beliefs such as cultural and vaccine hesitancy are also significant obstacles to the full immunization cover (Ali et al., 2019).

The case is made worse by the presence of malnutrition and under nutrition which conditions the immune system of children and predisposes them to frequent infections. The combined effect of adverse nutritional status and gastroenteritis results in an adverse outcome in the form of vicious cycle of illness and poor growth, stunting, underweight and development delays (Black et al., 2013). Pakistan Demographic and Health Surveys (PDHS) show that more than 38 percent of under ten children in Pakistan have stunted growth and about 17 percent of individuals are severely underweighted which portrays the inherent weakness of this group before infectious diseases such as gastroenteritis.

Moreover, climate change and seasonal change is a decisive factor in the transmission of gastroenteritis. Research indicates that elevated temperatures and rainwater in some months greatly enhance the contamination of water sources used to obtain drinking water resulting in peaks of gastroenteritis cases with the probability of more cases occurring during the summer and the monsoon seasons (Qureshi et al., 2021). Such times are also characterized by increased hospitalization of children and adults with the use of outpatient care in cases with acute diarrhea that create a significant load on the currently insufficient health care system.

Etiology of gastroenteritis is dictated not only by the kind of pathogen, but in addition, on the susceptibility of the host, the state of immunity, the population density, the area health infrastructure. Although viral pathogens, including rotavirus, norovirus, and adenovirus, are the most common causes among young children, it has become apparent over the past several years that sapovirus and astrovirus may be a common cause as well, especially in the areas where rotavirus vaccination has been implemented

in the majority of people (Oka et al., 2015; Murray et al., 2022). Such viruses are also very contagious and risk bringing about an outbreak, particularly in day cares, schools, and refugee camps where children can get in contact with one another.

Bacterial pathogens are actually less widespread in some areas of high incomes compared to those of the virus; however, they are extremely widespread in low- and middle-income countries because of unsafe food storage pantry, the employment of raw or undercooked animal materials, and the poor refrigeration. Interestingly, Campylobacter jejuni has emerged to be a major cause of bacterial gastroenteritis in most regions of Asia with incidences being linked to ingesting defiled chicken meat and raw milk (Kaakoush et al., 2015). Besides, resistant strains of Shigella and Salmonella, including those that secrete extended-spectrum beta-lactamases (ESBLs), are becoming an even more challenging therapeutic issue in pediatric groups (Ahmed et al., 2020).

Infections caused by parasites also play a major role in causing gastroenteritis in children especially in places with Currently poverty and poor hygiene have been blamed on parasitic infection among children. Giardia lamblia, Cryptosporidium parvum and Entamoeba histolytica are the most frequently involved protozoa and can cause persistent or even chronic diarrhea and result in children growth retardation and nutrients deficiency (Fletcher et al., 2012). These infections are oftentimes water related being transmitted by an intake of bad drinking water or recreational water supply. Parasitic gastroenteritis could go undiagnosed in a few cases either because of the lack of diagnostic facilities in rural health facilities.

Host factors are also important in defining the outcome and severity of gastroenteritis. An example is given by children whose immune systems have been weakened due to them having HIV/ AIDS, those receiving chemotherapy and those with congenital immunodeficiencies such that they are more susceptible to gastroenteritis that can be prolonged and severe in nature (Sanchez et al., 2016). Besides, the gut microbiome composition became a newly discovered important determinant of host resistance recently. The underrepresentation of microbial diversity in children caused by overuse of antibiotics or inadequate diet places children at a higher risk of enteric infection and recovery takes longer (Lozupone et al., 2012).

Seasonality and climate are environmental factors that also determine the cases and intensity of gastroenteritis. As one example, viral gastroenteritis is more common in cooler, drier months and bacterial and protozoal infections are more likely to occur in warmer and wetter months when bacteria thrives and there is a greater chance of water contamination during flooding and heavy rainfall (Ahmed et al., 2013; Levy et al., 2009).

The fact that gastroenteritis has a seasonality is a representation of the variations in both the environment and behavior which varies throughout the year. Along with summer and monsoon peaks, novel data are emerging to indicate that differences in food handling behavior, the dynamics of vectors (e.g., flies and cockroaches), and interpersonal transmission during the school months also contribute to the seasonality of infections (Nasrin et al., 2013). Increased microbial growth in food and moisture on food and water are observed during the warmer months, and the lack of refrigeration and hygiene also contribute to a pathogen in the form of E. coli, Salmonella, and Shigella in household and street vendors. The condition is aggravated among peri-urban and rural populations as lack of electricity to store perishable food substances and use of open water bodies that are easily polluted are common.

During monsoon season when the water levels invariably rise, excessive cross-contamination of sewage and drinking water is found, especially in zones with open run-offs near hand pumps or wells (Ali et al.,

2019). Not only do the spillages of the sewages contaminate the ground water sources, but also raises the level of vector breeding worsening the chances of various infectious diseases contracted, among them being gastroenteritis. The increased demand during these peak seasons normally overwhelms the health systems in rural areas such as Mardan that tend to give way to delayed treatment of the children and increase in complication rates.

In addition to infrastructure, seasonal seasonal behavioral patterns are also inherent factors in how the diseases could be spread. Winter time, when bacterial gastroenteritis is more likely to decrease, the prevalence of viral gastroenteritis--in particular norovirus and rotavirus--increases. It is especially true in enclosed indoor environments (e.g. schools or childcare facilities) where there is close contact that facilitates a high rate of transmission between people (Ahmed et al., 2014; Atmar & Estes, 2006). The challenge with norovirus in these settings is that outbreaks are frequently hard to contain because of the stability of the virus on surfaces and these virus's low infectious threshold dose.

Water, Sanitation, and Hygiene (WASH) issue are always considered when the gastroenteritis remains a topical issue in District Mardan. Besides the insufficient, safe drinking water, just under half of KP households in rural areas have access that is improved (between 42% and 56%), whereas less than 30 percent comply with good handwashing with soap throughout crucial moments like, after a defecation session, like before a meal (Pakistan Bureau of Statistics, 2021; UNICEF, 2021). This low compliance with hygiene measures poses a high chance of fecal-early transmission of pathogens and among young children, who are more likely to place objects in their mouths.

The impact of educational status of caregivers in particular mothers in exposing a child to gastroenteritis and combating its effects is very high. Mother with a higher level of education will be in a better position to ensure earlier signs of illnesses, uphold hygiene, offer weaning foods within safety measures and ensure medical attention at the right time (Bhutta et al., 2010). Sadly, in several sections throughout District Mardan women literacy levels are low, and this restricts the scope of transfer of knowledge on child health. Also, the role of gender can exist in the absence of women to choose healthcare services to their children.

The nutritional status is another compounding factor. Gastroenteritis is easier to catch in the malnourished children, and frequent diarrhea, in its turn, contributes to additional nutriment losses and the development of a vicious cycle of infection and malnutrition. The common Zinc and vitamin A deficiencies among the KP children in Khyber Pakhtunkhwa considerably suppress mucosal immune system making diarrhea diseases more severe and prolonged (Walker et al., 2007). These indicate the need to include nutrition-based interventions in gastroenteritis management programs, such as supplement, school-based feeding program, and nutrition education to the mothers.

Various research studies carried out in Pakistan have indicated that children at the ages below ten years are most vulnerable to gastroenteritis. Such reasons attributed to this include their unfavorable immune systems, non-exclusive breastfeeding, and exposure to dirty environs. According to one study in Lahore, viral gastroenteritis caused almost 45 percent of pediatric hospitalizations regarding diarrhea, and a considerable proportion of them were tested positive in regards to rotavirus infection (Ali et al., 2019). Moreover, malnutrition as a typical problem of rural regions of Khyber Pakhtunkhwa disrupts the ability of immunity systems and makes people vulnerable to infections, such as gastroenteritis (Black et al., 2013).

The case in district Mardan is a fast-urbanizing district comprising of both urban and rural residents. Another large group of people continue to use unfiltered groundwater, and this unfiltered water may

include cholera because proper garbage collecting systems are not in place. Moreover, children are particularly susceptible to gastrointestinal infections since several people are not aware of the need to wash their hands with soap (Khan et al., 2020).

That is why this study shall seek to address this gap as it shall determine the prevalence of gastroenteritis in children in the District of Mardan as well as the risk factors thereof and determine the seasonal effects on prevalence of the disease. The results will help to have a wider picture of the malady and to plan specific measures of intervention, particularly at risk in rural communities.

2. Materials and methods

2.1 Study design

This cross-sectional in District Mardan, in Khyber Pakhtunkhwa province of Pakistan. The data was collected from June 2024 to May 2025 from children below ten years of age who were present in healthcare institutions with clinical manifestations that indicated that the children could have gastroenteritis. These are manifested as diarrhea (with or without bleeding and/or mucus), vomiting, fever and dehydration. Only those who met the inclusion criteria were enrolled. Children who had chronic gastrointestinal conditions, used antibiotics (within 2 weeks) or other diseases were not considered because they may confound this study. It was done by seeking written informed consent of the parents or guardians before samples and data were collected as per the ethical rules of research. The number of the participants involved in this study was 200 to 300 individuals chosen with the help of stratified random sampling.

The questionnaire included variables such as age, gender, residence (urban/rural), family income, parental education, and hygiene-related behaviors (e.g., handwashing, access to clean drinking water, and sanitation facilities). Clinical information regarding the type and duration of diarrhea, vomiting, fever, and dehydration was also recorded. This information was used to assess risk factors associated with gastroenteritis in the study population.

Fresh stool samples were collected from each participant using sterile, leak-proof containers. Each sample was appropriately labeled with a unique identification code. All samples were transported to the microbiology laboratory within 2–4 hours of collection in cooled iceboxes to maintain the integrity of the specimen. Upon arrival at the laboratory, samples were immediately processed for both microbiological and molecular analyses.

2.2 Laboratory analysis

Stool samples were collected in sterile, leak-proof containers from children presenting with symptoms of gastroenteritis. Each sample was labeled and documented using a unique identification code. The samples underwent both macroscopic and microscopic examinations. In the macroscopic examination, the stool was assessed for color, consistency (watery, semi-formed, formed), and the presence of blood, mucus, or pus. For the microscopic examination, a small portion of the stool sample was placed on a glass slide and mixed with a drop of normal saline for wet mount preparation. Another slide was prepared using iodine solution to enhance parasite detection. The slides were covered with cover slips and observed under a compound microscope at 10x and 40x magnifications. This examination aimed to detect the presence of white blood cells (WBCs) and red blood cells (RBCs), as well as parasitic ova, cysts, and trophozoites. To confirm the presence of inflammatory cells, additional smears were stained using methylene blue stain and examined under oil immersion (100x objective).

Stool is cultured by the preparation of selective and differential media under sterile conditions. The used is MacConkey agar, Salmonella-Shigella (SS) agar, and Xylose Lysine Deoxycholate (XLD) agar. When culturing bacteria, fresh stool samples using sterile containers are placed on the prepared agar plates with the help of a sterile loop. Inoculated plates are subsequently incubated

at 37 oC in an aerobic incubator at 18-24 hours. After incubation, the plates are examined for the growth of distinctive bacterial colonies. *Salmonella spp.* typically produce colorless colonies on MacConkey agar and black-centered colonies on SS or XLD agar due to hydrogen sulfide production. *Shigella spp.* form colorless colonies without black centers, while *Escherichia coli* (E. coli) produce pink colonies on MacConkey agar due to lactose fermentation. The morphological characteristics of these colonies serve as preliminary indicators of bacterial identity.

For molecular analysis, the DNA is extracted from Cultured Bacteria Using Commercial Kit "Thermo Scientific Gene JET Genomic DNA Purification Kit". For the PCR amplification Thermo scientific DreamTaqtm Green PCR Master Mix (2X) is used. Primer used for species amplification are given in Table 1.

Table 1: Primer Table for PCR Detection of Pathogenic Bacteria

Bacterial Species	Target Gene	Primer Name	Sequence (5'–3')	Amplicon Size
Salmonella spp.	invA	InvA-F / InvA-R	F: GTGAAATTATCGCCACGTTCG R: TCATCGCACCGTCAAAGGAACC	~284 bp
Shigella spp.	ipaH	ipaH-F / ipaH-R	F: GTTCCTTGACCGCCTTTCCGATAC R: GCCGGTCAGCCACCCTCTGAGAGT	~619 bp
Escherichia coli (EPEC)	eaeA	eaeA-F / eaeA-R	F: GACCCGGCACAAGCATAAGC R: CCACCTGCAGCAACAAGAGG	~384 bp

2.3 Data analysis

Data were entered and analyzed using SPSS (Statistical Package for the Social Sciences) version 4.4.8. Descriptive statistics, including frequencies and percentages, were used to estimate the prevalence of gastroenteritis and associated pathogens. Logistic regression was applied to assess the association between various risk factors (e.g., hygiene practices, socioeconomic conditions) and the occurrence of gastroenteritis. Seasonal trends in gastroenteritis incidence were analyzed using time-series analysis, allowing the identification of peak periods and seasonal patterns. A p-value of less than 0.05 was considered statistically significant.

3. Result

3.1. Sociodemographic characteristics

Out of the total 250 children enrolled in the study, 132 (52.8%) were males and 118 (47.2%) were females as mention in table 4.1. To statistically assess whether the observed difference in gender distribution is significant, a Chi-square (χ^2) test was applied. The resulting p-value was 0.041, which is less than 0.05, indicating a statistically significant difference in the gender-wise prevalence of gastroenteritis among the study population.

Table 2: Gender Distribution of Study Population

Gender	Frequency (n)	Percentage (%)	Statistical analysis
Male	132	52.8	P<0.041
Female	118	47.2	
Total	250	100	

Children in the study were divided into four age categories: <1 year, 1–3 years, 3–5 years, and >5–10 years. The results revealed that the highest proportion of gastroenteritis cases occurred in the 1–3 years age group, with 105 children (42%) affected, followed by the 3–5 years group with 83 children (33.2%), <1 year with 39 children (15.6%), and the lowest prevalence in the >5–10 years category with 23 children (9.2%) as shown in table 4.2. This age-related trend suggests that children in early childhood, especially those aged 1–3 years, are at a higher risk of gastroenteritis.

Table 3: Positive and Negative Cases in Age Groups

Age Group	Total	Positive Cases	Negative Cases	Percentage (%)	Statistical analysis
<1 year	39	28	11	15.6	P<0.028
1–3 years	105	91	14	42.0	
3–5 years	83	70	13	33.2	
>5–10 years	23	15	8	9.2	

Higher number of children belonged to rural areas (150 children, 60%) compared to urban areas (100 children, 40%) (Fig 4.3 & table 4.3). This indicates a higher

prevalence of gastroenteritis among children living in rural regions. Several underlying factors may contribute to this observation, including limited access to clean drinking water, poor sanitation infrastructure, inadequate healthcare services, and low levels of education and health awareness in rural communities. These conditions create an environment conducive to the transmission of gastrointestinal pathogens. The Chi-square (χ^2) test yielded a p-value of 0.041, indicating a statistically significant association between area of residence and gastroenteritis prevalence ($p < 0.05$).

Table 4: Area-wise Distribution of Study Population and Gastroenteritis Cases

Residence Type	Total cases	Positive Cases (n)	Negative Cases
Rural	150	115	35
Urban	100	63	37

The socioeconomic status of the children's families was categorized into low-income, middle-income, and high-income groups. Among the 250 children, 169 (67.6%) belonged to low-income families, while 81 (32.4%) were from middle-income households, with no representation from high-income families. A total of 122 (table 4.4) positive gastroenteritis cases were observed in the low-income group compared to 38 in the middle-income group, indicating that children from low-income backgrounds were more affected.

Table 5: Distribution of Gastroenteritis Cases by Socioeconomic Status

Socioeconomic Status	Total Cases	Positive Cases	Negative Cases	Percentage of Total (%)	Statistical analysis
Low Income	169	122	47	67.	P< 0.019
Middle Income	81	38	43	32.4	
High Income	0	0	0	0	

Parental education is a significant determinant of child health. In this study, 73.6% of mothers and 61.2% of fathers had no formal education, indicating low awareness regarding hygiene and healthcare practices, which may increase the risk of gastroenteritis among children.

Table 6: Parental Education Level

Parent	Education Level	Frequency (n)	Percentage (%)
Mother	No Education	184	73.6%
	Primary/Secondary	66	26.4%
Father	No Education	153	61.2%
	Primary/Secondary	97	38.8%

3.2. Clinical features observed

Gastroenteritis in children typically presents with a range of gastrointestinal and systemic symptoms that vary in severity and duration. In this study, all 250 children diagnosed with gastroenteritis experienced diarrhea, making it the most common symptom. Many of them also suffered from vomiting (72%) and fever (68%), while more than half (55.2%) showed signs of moderate to severe dehydration as shown in table 4.6. These symptoms reflect the rapid fluid loss and physical stress caused by the infection, especially in young children whose immune systems are still developing.

Some children had more severe signs—nearly 38% had mucus in their stool, and about 11% had blood, indicating possible bacterial infections like dysentery. On average, the illness lasted between 2 to 6 days. These results underline the need for quick medical attention, proper hydration, and better awareness of hygiene to prevent the spread and impact of gastroenteritis in children.

Table 7: Clinical Features Observed in Children with Gastroenteritis (N = 250)

Clinical Feature	Positive Cases	Negative Cases	Percentage (%)
Diarrhea	250	0	100
Vomiting	180	70	72.0
Fever	170	80	68.0
Moderate to Severe Dehydration	138	112	55.2
Mucus in Stool	94	156	37.6
Blood in Stool	28	222	11.2
Duration of Symptoms	2–6 days	—	—

3.3. Microscopic examination of stool samples

The stool samples from 250 children were subjected to both macroscopic and microscopic examination to identify features consistent with gastroenteritis and to detect possible infectious agents.

On gross examination, a large proportion of the stool samples (58%) were watery in consistency, which is typical in acute gastroenteritis, often caused by viral or bacterial agents. About 32% of the samples were semi-formed, indicating mild or recovering diarrhea, while 27% showed visible blood and/or mucus. The presence of blood and mucus is typically associated with invasive bacterial infections or protozoan pathogens like *Entamoeba histolytica*.

White blood cells (WBCs) were observed in 183 samples (73%), which strongly indicates an inflammatory or infectious process. Red blood cells (RBCs) were found in 53 samples (21%), suggesting mucosal damage and potential dysentery. Parasitic forms, such as ova, cysts, or trophozoites, were detected in 35 samples (14%). The most frequently identified protozoa were *Giardia lamblia* and *Entamoeba histolytica*. These findings were confirmed using the formalin-ether concentration technique, especially in samples where the parasite load was initially low, enhancing the sensitivity of detection (Table 1).

Table 8: Stool Sample Analysis (Macroscopic and Microscopic Findings, N = 250)

Observation	Positive Cases (n)	Negative Cases (n)	Percentage (%)
Macroscopic Examination			
Watery Consistency	145	105	58.0%
Semi-formed Stools	80	170	32.0%
Blood and/or Mucus Present	68	182	27.2%
Microscopic Examination			
White Blood Cells (WBCs)	183	67	73.2%
Red Blood Cells (RBCs)	53	197	21.2%
Parasitic Ova/Cysts/Trophozoites	35	215	14.0%
Giardia lamblia / Entamoeba histolytica	35	215	14.0% (confirmed)

3.4. Bacterial culture results

To identify the bacterial pathogens responsible for gastroenteritis in children, all 250 stool samples were subjected to culture on selective and differential media. The results of the culture revealed the presence of three major enteric bacterial pathogens: *Salmonella spp.*, *Shigella spp.*, and *Escherichia coli* (specifically enteropathogenic *E. coli* or EPEC).

Salmonella spp. were isolated in 52 out of 250 cases, accounting for 20.8% of the total. These organisms are common causes of foodborne illnesses in developing regions. On Xylose Lysine Deoxycholate (XLD) agar and Salmonella-Shigella (SS) agar, Salmonella colonies appeared with black centers, indicative of hydrogen sulfide (H_2S) production—a hallmark for their preliminary identification. Shigella spp. were found in 33 samples, representing 13.2% of cases. *Shigella* typically causes bacillary dysentery and is highly contagious, especially among children in crowded or unsanitary environments. On selective media, these bacteria formed colorless colonies without H_2S production on XLD and SS agar, aiding in differentiation from *Salmonella*. Enteropathogenic Escherichia coli (EPEC) were isolated in 47 cases (18.8%). EPEC strains are major contributors to infantile diarrhea in developing countries. These bacteria formed pink colonies on MacConkey agar, indicating lactose fermentation, a classic trait of *E. coli*. (Table 9)

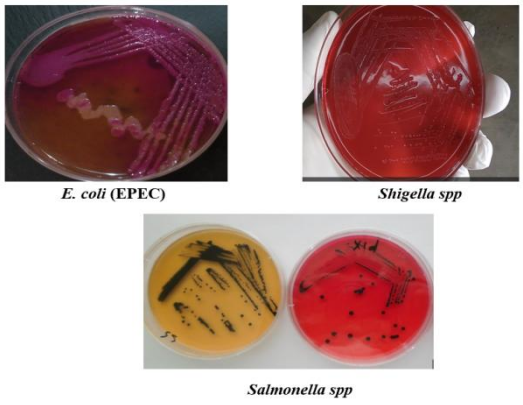

Figure 1: culture result of different spices

Table 9: Bacterial Pathogens Isolated from Stool Samples (N = 250)

Bacterial Pathogen	Positive Cases (n)	Negative Cases (n)	Percentage (%)	Selective Media Characteristics
Salmonella spp.	52	198	20.8	Black-centered colonies on XLD/SS agar (H_2S positive)
Shigella spp.	33	217	13.2	Colorless colonies on XLD/SS agar (H_2S negative)
E. coli (EPEC)	47	203	18.8	Pink lactose-fermenting colonies on MacConkey agar

3.5 Molecular confirmation by PCR

Polymerase Chain Reaction (PCR) was employed as a confirmatory method to accurately identify bacterial isolates obtained through culture, enhancing detection precision, particularly where traditional culture methods may yield inconclusive results due to contamination or poor growth. In this study, DNA was extracted from all culture-positive samples of *Salmonella spp.*, *Shigella spp.*, and enteropathogenic *E. coli* (EPEC), using species-specific primers targeting conserved genes: *invA* for *Salmonella* (~284 bp), *ipaH* for *Shigella* (~619 bp), and *eaeA* for EPEC (~384 bp). PCR analysis demonstrated high sensitivity and specificity, confirming 49 out of 52 *Salmonella* isolates (94.2%), 31 out of 33 *Shigella* isolates (93.9%), and 45 out of 47 EPEC isolates (95.7%) as shown in table 4.9. The amplified products were visualized on agarose gel electrophoresis under UV transillumination, showing distinct bands corresponding to the expected product sizes, thus confirming the presence and identity of each pathogen. These findings highlight the reliability of culture methods when validated through PCR and emphasize the value of molecular tools in clinical diagnostics and epidemiological investigations.

Table 10: Molecular Confirmation of Bacterial Isolates by PCR

Bacterial Species	Target Gene	PCR Product Size (bp)	Culture Positive (n)	PCR Positive (n)	Confirmation Rate (%)
Salmonella spp.	invA	~284 bp	52	49	94.2%
Shigella spp.	ipaH	~619 bp	33	31	93.9%
E. coli (EPEC)	eaeA	~384 bp	47	45	95.7%

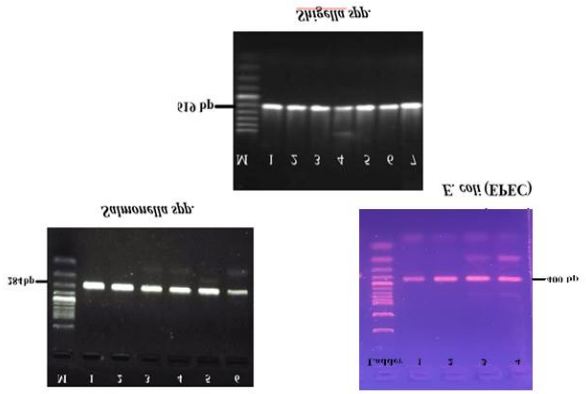

Figure 2: Molecular Confirmation of Bacterial Isolates by PCR

3.6 Seasonal variation of gastroenteritis cases

The importance of seasonal variation can be observed in the epidemic of gastroenteritis especially in locales where hygienic, access to water resources and climatic conditions vary during different seasons. In this paper, there was a distinct seasonal trend found where the highest rate of gastroenteritis cases was reported during the summer season (June to August) representing 38.4 percent of all the cases. This is peak season and the time when there is the highest temperature and large use of contaminated source water that helps in raising the spread of the gastrointestinal pathogens. Then came the monsoon season (September to November) which showed cases of 29.2 percent following the monsoon as it is through stagnant water, bad sanitation and flooding that causes waterborne infections. The highest percentages occurred in winter (December to February), which constituted 18 percent of the cases and the lowest prevalence was noted in the season of spring (March to May) which was 14.4 percent. This shift indicates that environmental factors, the quality of water and food handling procedures also play an important role in gastroenteritis transmission prompting majority of health protection interventions during warmer and wetter weather intensification.

Table 11: Seasonal Distribution of Gastroenteritis Cases

Season	Frequency (n)	Percentage (%)
Summer (Jun–Aug)	96	38.4
Monsoon (Sep–Nov)	73	29.2
Winter (Dec–Feb)	45	18.0
Spring (Mar–May)	36	14.4

3.7 Risk factor analysis for gastroenteritis

Both univariate and multivariate logistic regression analyses were used to perform a risk factor analysis in order to determine the presence of significant associations between the variables studied in this research and gastroenteritis in children. The findings illuminated a number of elements that contributed to a major part in disease prevalence. Lowest handwashing before a meal was the most significantly related which gave an odds ratio (OR) of 3.1 and a 95 percent confidence interval (CI) of 1.95 to 5.0 showing that children whose families were not keen on following hand hygiene were more than three times more likely to have experienced gastroenteritis. Equally, utilization of untreated drinking water (OR = 2.7; CI: 1.6 4.5) and poor sanitation (OR = 2.3; CI: 1.3 3.8), revealed how the environmental hygiene plays a pivotal role. The probability of infection was also significantly higher when parents were poorly educated (OR = 2.0; CI: 1.2 3.3) which shows the great role awareness and health literacy play in prevention of diseases. The rural population OR was 1.8 (CI: 1.1-2.9) and the authors indicated that these rural children were more vulnerable because the infrastructure and public health services were limited. Conversely, gender (p = 0.23) and household income level (p = 0.08) was not statistically significant but data tend to indicate that those with low household income levels were at greater risk. These results highlight the need to emphasize on behavioral and environmental interventions to alleviate gastroenteritis burden.

Table 12: Risk Factor Analysis for Gastroenteritis

Risk Factor	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
Lack of handwashing	3.1	1.9–5.0	<0.001
Untreated drinking water	2.7	1.6–4.5	<0.001
Poor sanitation facilities	2.3	1.3–3.8	0.002
Low parental education	2.0	1.2–3.3	0.005
Rural residence	1.8	1.1–2.9	0.021
Gender	1.2	0.8–1.9	0.230
Low household income	1.5	0.9–2.3	0.080

4. DISCUSSION

Gastroenteritis has been a disease of great morbidity and death in children especially in the developing nations such as Pakistan. The present paper based on the District Mardan investigates extensive features of this illness such as prevalence, risk aspects, symptomology, seasonality, and pathogenic microbes. Notably, the paper leveraged complementary techniques in diagnostic methods traditional and molecular diagnostics; therefore, giving a solid reflection on the issue of gastroenteritis in children in this area.

In the age wise analysis, an estimated prevalence of 42% was found in children aged 1 3 years and 3 5 years (33.2%). Children of this age are characterized by developing immunity and prevalent exposure to pathogens because of oral exploratory behavior. These outcomes are consistent with those of Liu et al. (2012) and Walker et al. (2013), citing immature immunity and poor diversity of the gut microbs as a possible causal factor. This age-specific vulnerability is again confirmed by studies done by Lanata et al. (2013), Kotloff et al. (2013) on South Asian and African populations.

Environmental factors were highly influential. It was high in the rural area (60%) in line with the findings of Nasrin et

al. (2013) and Alam et al. (2014), where poor water sanitation and hygiene (WASH) practices in the rural communities were cited as the first key factors. These findings have been supported by a 2020 report by WHO, which observed that more than 40 percent of households in rural areas in Pakistan did not have access to clean drinking water. The same patterns are said by Pathela et al. (2006) in Bangladesh and Ejemot-Nwadiaro et al. (2015) in Nigeria.

Children from low-income families (67.6%) exhibited significantly higher infection rates. These outcomes align with findings by Checkley et al. (2004) and confirmed by Troeger et al. (2017), who reported that low income correlates with inadequate access to sanitation, education, and healthcare, increasing disease vulnerability. Research by Humphrey (2009) further explains that undernutrition exacerbated by recurrent infections leads to a vicious cycle in impoverished settings.

Parental education, particularly maternal education, showed strong correlation with disease occurrence. In our study, 73.6% of mothers and 61.2% of fathers of infected children were uneducated. These data resonate with findings by Bartram & Cairncross (2010) and Black et al. (2010), who stressed the impact of education on hygiene practices and healthcare decisions. A similar correlation was demonstrated by Fink et al. (2011) across several low-income countries.

Clinically, diarrhea was the universal symptom, with high frequencies of vomiting (72%), fever (68%), and dehydration (55.2%). These symptoms reflect acute gastroenteritis, as reported in studies by Kotloff et al. (2013) and Ahmed et al. (2009). Bloody and mucoid stools, observed in 11.2% and 37.6% of cases respectively, indicate invasive pathogens such as Shigella or E. histolytica, consistent with studies by Farthing et al. (2009) and Haque et al. (2006).

Microscopic analysis identified WBCs in 73% and RBCs in 21% of samples, indicating inflammatory or invasive infections. Parasitic pathogens like Giardia lamblia (14%) and Entamoeba histolytica (14%) were common, matching prevalence data from Akhtar et al. (2010) and WHO regional estimates. These findings are further substantiated by similar research conducted in Afghanistan and India where protozoal infections contributed significantly to pediatric gastroenteritis.

Since PCR-based detection of invA (Salmonella), ipaH (Shigella), and eaeA (EPEC) based molecular identification has high specificity, there was strong agreement with culture-based results. This justifies the usefulness of molecule diagnostics in low resources. Chaudhry et al. (2014) and Kabayiza et al. (2014) highlighted the advantage of PCR to the detection of mixed infection and fastidious organisms that are easily invisible in a culture.

As visioned by seasonal analysis, the highest incidence was seen in the summer (38.4%) and monsoon period (29.2%) which correlates with more human exposure to waterborne pathogens during hot summer months and the monsoons. Similar findings were obtained in the studies conducted by Gupta et al. (2007) and Ahmed et al. (2013), where a more influential effect of diarrhea occurred in warm and rainy months.

The following identified risk factors were statistically significant; absence of handwashing (OR = 3.1), poor sanitation, and unsafe drinking water. These are the similar observations that are voiced by WHO guidelines (2019) and UNICEF WASH reports that mention these factors as the main contributors to diarrheal diseases. Curtis and Cairncross (2003) and Luby et al. (2005) also reported similar risk profiles in several countries across the world.

Where required comparative studies have been conducted in the region, this research is clearly in line with data on the same set of parameters, namely child diarrhea in Bangladesh, Nepal, and Afghanistan, where poverty, education and sanitation play a major role. Specifically, the GEMS and MAL-ED studies (Nasrin, et al, 2013; Platts-Mills, et al, 2018) found similarly

patterned pathogen prevalence and risk factors. Our demographic and environmental determinants also have supporting evidence of multi-country reviews by the GBD Diarrhoeal Collaborators (2017).

To sum it up, gastroenteritis in the District Mardan is a multifactorial health issue that is being affected by biological, environmental, and socioeconomic parameters. The study demonstrates the need to enhance maternal education, sanitation, availability of clean water, and early diagnosis by applying molecular techniques. The policy implications can be seen in the form of strengthening the primary health care, local sensitization programs, and customized health initiatives in order to reduce the spread of the disease. Future work may be based on vaccine 2 efficacy, nutrition interventions and antimicrobial resistance profile of the paediatric population.

5. Conclusion

The current study demonstrates a high burden of gastroenteritis in children across District Mardan, with peak prevalence in the age groups 13 years and the presenting ages range between 1 and 3 years old and in rural, low-income households. The results highlight the importance of poor hygiene, untreated water and low education of parents among other environmental, behavioral, and socioeconomic factors in the transmission of the disease. The common clinical symptoms included diarrhea, vomiting and dehydration and the commonly isolated and confirmed pathogenic bacteria included Salmonella spp, Shigella spp and EPEC confirmed by PCR. These seasonal patterns, especially during the summer and monsoons, lend further weight to the necessity of proper, seasonal, public health action. In summary, culture, molecular diagnostics, and risk factor analysis provide a sound insight into epidemiology of pediatric gastroenteritis in this region.

Ethical statement

This research was conducted following the ethical guidelines set forth by the Declaration of Helsinki. Informed consent was obtained from the participants, detailed information was provided about the study's purpose, procedures, and their rights; their participation was entirely voluntary. The confidentiality of the participants was upheld throughout the study. the study protocol was approved by the Advanced Study Research Board (ASRB /Dir/A&R/AWKUM/2025/1709) committee members of Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan.

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