

## ORIGINAL ARTICLE

**Prevalence, Risk Factors and Patterns of Different Underlying Enteropathogens in Pediatric Patients**FARMAN ULLAH<sup>1</sup>, TAJ MUHAMMAD<sup>2</sup>, RIDA NAZ<sup>3</sup><sup>1</sup>Associate Professor, Department of Pediatrics, Gomal Medical College Dera Ismail Khan<sup>2</sup>Assistant Professor, Department of Pediatrics, Gomal Medical College Dera Ismail Khan<sup>3</sup>Regional Blood Centre, Dera Ismail KhanCorrespondence to: Rida Naz, Email: [dr.ridaanaz@gmail.com](mailto:dr.ridaanaz@gmail.com)**ABSTRACT****Background:** According to the WHO, diarrhea is a result of inadequate water, sanitation, and hygiene and is the second leading cause of death in pediatrics.**Objectives:** Therefore, the study was intended to discover enteropathogens linked with diarrhea in pediatrics less than 5 years, their prevalence, distribution patterns and risk factors.**Methods:** The study was conducted in selected hospitals of District Dera Ismail Khan from March 2021 to October 2022 and comprised 384 patients diagnosed with bacterial (through culture) and parasitic (through microscopic examination) agents. Their demographic characteristics and risk factors were recorded on the pre-designed questionnaire.**Results:** Out of 384, 123 patients (32.03%) were suffering from bacterial diarrhea ( $p < 0.05$ ) and 261 children (67.96%) were diagnosed with parasitic ailments. Among bacterial causes, *Escherichia coli* (49.78%) was predominant ( $p < 0.05$ ), followed by *Salmonella* (18.92%), *Campylobacter* (10.66%), *Shigella* (7.13%) and *Vibrio* (1.91%), while the major parasitic infestation ( $p < 0.05$ ) involved *Giardia* (21.12%), *Ascaris* (17.78%), hookworms (14.39%), *Trichuris* (12.98%) *Entamoeba* (12.84%) and *Taenia* (3.19%). Most infected children belonged to the rural population (59.11%) than the urban (40.88%).**Practical implications:** The current study will pave a path for the clinicians to differentially diagnose the bacterial and parasitic origin enteropathogens in pediatric patients.**Conclusion:** Most of the diarrheic patients were diagnosed with parasitic than bacterial etiological agents. The major risk factors of the infection were poor hygiene, sanitation, weak immunity and playing of children in the contaminated soil and ingesting contaminated food materials.**Keywords:** Acute diarrhea; Parasites; Poor hygiene; Stool culture**INTRODUCTION**

Infectious diarrhea is a major issue since they cause three million deaths and substantial morbidity globally per annum. In 2015, diarrhea was the 9<sup>th</sup> highest cause of mortality worldwide, accounting for over 1.3 million deaths annually, and the 4<sup>th</sup> leading cause of death in children under five years of age, with approximately 499,000 deaths. According to the WHO, Africa and South-East Asia account for 78% of all diarrheal infant fatalities in poor countries. Poor environmental circumstances, socioeconomic level and behavioral characteristics are all substantially connected with the probability of transmission of diarrhea<sup>1</sup>. According to the onset and extent, diarrhea is classified into acute (<7 days), prolonged (7–14 days) and chronic (>14 days). The etiology of chronic diarrhea varies by child's age, socioeconomic circumstances, immunological condition and clinical context. Enteric infections are the most common cause of chronic diarrhea and subsequent infections with the same or different bacteria may be responsible for the persistence of symptoms<sup>2</sup>.

In developing countries, *Salmonella*, *Yersinia enterocolitica*, *Proteus*, *Vibrio cholerae*, *Campylobacter*, *Escherichia coli*, *Shigella* and viruses are the primary causes of infantile acute diarrhea<sup>3</sup>. Globally, gastrointestinal infections caused by *Shigella* species are a significant source of mortality and morbidity in children below 5 years<sup>4</sup>. *Shigella* species are associated with numerous sources, including ingestion of raw meat, unsanitary food handling techniques, dangerous water supplies, extended field slaughtering and unhygienic. These are disseminated from the affected people through the oro-fecal route<sup>5</sup>. Parasitic infections associated with diarrhea include *Ascaris lumbricoides*; *Giardia lamblia*, *Hymenolepis nana*, *Entamoeba histolytica*, *Cryptosporidium*, *Blastocystis hominis*, *Trichuris trichiura*, and *Cyclospora cayentanensis*<sup>1, 6</sup>. Rotavirus is the most prevalent viral cause of gastroenteritis associated with severe diarrhea, which kills 400,000 children per annum throughout the globe<sup>7-9</sup>.

Besides it, child malnutrition remains a significant risk factor for both death and impaired long-term development. In these conditions, early exposure to enteropathogens has been linked to poor child growth. The majority of previous studies have evaluated a limited number of pathogens and found associations with

*Shigella* and enterotoxin-producing *Escherichia coli*, *Campylobacter*, *Cryptosporidium*, *Giardia*, and *Ascaris*. The development of highly sensitive molecular diagnostics for a broad spectrum of enteropathogens has brought fresh insight into the pathogenesis of diarrhea in low-resource settings in children<sup>3, 7</sup>.

*Shigella*-caused diarrhoea remains a significant public health hazard, particularly among children. The burden, antibiotic resistance patterns, and related risk factors for *Shigella* infections, was investigated in a routine surveillance system. The prevalence, antimicrobial susceptibility patterns, and related risk factors of *Shigella* infections among diarrheic paediatric patients were reported in the city of Gondar<sup>4</sup>.

Other than rotavirus, *Entamoeba histolytica*, an intestinal protozoan parasite, is associated with diarrheal disorders, particularly human amoebiasis, which is primarily a worldwide health concern in developing nations. It is the largest cause of death from parasites worldwide and is responsible for more than 50 million cases of infection annually, of which 40,000–110,000 patients die. In fact, the National Institute of Health and Infectious Diseases in the United States ranked *E. histolytica* as the parasite with the second-highest priority. 22.3% of patients hospitalized with AGE were infected with *E. histolytica*, according to a prior study conducted in Beirut, Lebanon<sup>8</sup>.

Therefore, this research was intended to discover enteropathogens linked with diarrhea in pediatrics less than 5 years, their prevalence, distribution patterns and risk factors.

**MATERIALS AND METHODS**

**Study Design and Location:** The cross-sectional study was conducted in the selected three tertiary care hospitals and seven pediatric clinics of District Dera Ismail Khan from March 2021 to October 2022, for the investigation of the prevalence of enteropathogens in pediatrics and associated risk factors. A standardized questionnaire was used to collect data on general socio-demographic variables and acute diarrhea exposure factors, and fecal samples were taken from each patient for examination along with detailed clinical data from patient records.

**Sample Size:** The sample size was estimated using a single population proportion calculation with 5% predicted margins of

error, a 95% confidence interval, and a 10% non-response rate. The ultimate sample size was 384<sup>9</sup>.

**Inclusion and Exclusion Criteria:** The study included participants under 5 years of age, who were recruited while they sought treatment for acute diarrhea at the Pediatric Outpatient Department and hospitalized children with diarrhea and gastrointestinal infections, diagnosed with enteropathogens. While, exclusion criteria comprised the children with chronic diarrhea, malnutrition, immunodeficiency and structural deformities which could distort the interpretation of our results<sup>8</sup>.

**Stool Sample Processing:** The caregivers were directed to collect their child's morning stool specimen in a sterile fecal container for above 2 years child and children younger than 24 months were given a diaper. The collected stool samples (each morning), were stored in an ice box with ice packs and were then analyzed in the microbiology laboratory through stool culture techniques (Figure A). Using Kato Katz techniques, helminths were identified in the stool samples. After preparing duplicate thick smears of each stool sample, two laboratory personnel independently examined the slides for helminth eggs using microscopy<sup>10</sup>.



Figure A: Stool culture on MacConkey agar

**Assessment of Risk Factors:** A standardized questionnaire was presented to the child's parent or guardian in order to obtain data on socio-demographic characteristics and potential infection risk factors. All the clinical, microbiological and pathological investigations were recorded and analyzed for the determination of the potential risk factors for pediatric gastroenteritis.

**Statistical Analysis:** SPSS software version 20 was used for data statistical analysis. At  $p < 0.05$ , statistically significant connections were stated. Child and household characteristics, as well as the child's health status, inflammation, micronutrient deficiencies and signs of sickness, were characterized using descriptive statistics. We determined the prevalence of each pathogen in the feces of each child and the number of infections discovered per child. A priori, the child's gender and age in months were included in the adjusted models as potential confounders.

**Ethical Approval:** Prior to collecting any data for the research, proper ethical approval was obtained from the Ethical committees

of the concerned health institutes and the confidentiality of the patients and their family were maintained according to prevailing government policies.

**RESULTS**

The cross-sectional study was conducted in District Dera Ismail Khan from March 2021 to October 2022, for the investigation of the prevalence of enteropathogens in 384 pediatric patients. It was found that 123 of the pediatric patients (32.03%) were suffering from acute diarrhea caused by bacterial infections, while the rest of the 261 children (67.96%) were diagnosed with parasitic ailments ( $p < 0.05$ ) underlying the diarrhetic conditions (Table 1). The stool sample of the patients diagnosed with bacterial diarrhea was cultured on agar media and the basis of morphology and colonial characteristics, the genera of the bacteria were determined and it was found that most of the bacterial diarrhetic cases were caused by *Escherichia coli* (49.78%) ( $p < 0.05$ ), followed by *Salmonella* (18.92%), *Campylobacter* (10.66%), *Shigella* (7.13%) and *Vibrio* (1.91%) (Figure 1). The stool sample of the patients diagnosed with parasitic diarrhea was examined microscopically using direct microscopy and floatation techniques and was differentially diagnosed based on the morphology of their eggs and cysts. It was found that the major parasitic infestation ( $p < 0.05$ ) involved in acute diarrhea in children was *Giardia* (21.12%), followed by *Ascaris* (17.78%), hookworms (14.39%), *Trichuris* (12.98%) *Entamoeba* (12.84%) and *Taenia* (3.19%), while 17.7% of the stool samples revealed mixed worm infestation (Figure 2). The clinical manifestations of bacterial and parasitic diarrhea were comparatively studied and it was found that bacterial diarrhea was mostly ( $p < 0.05$ ) characterized by vomiting, nausea, anorexia, weakness, dehydration, etc, while parasitic diarrhea was clinically manifested with ( $p < 0.05$ ) flatulence, colic, weakness, dehydration, abdominal discomfort and nausea (Figure 3).

Table 1: Overall prevalence of bacterial and parasitic ailments in acute diarrhea

No. of Patients (n)	Patients diagnosed with bacterial infections n(%)	Patients diagnosed with parasitic infections n(%)	p-value
384	123 (32.03)	261 (67.96)	0.00001*

\*indicated the value is significant at  $p < 0.05$

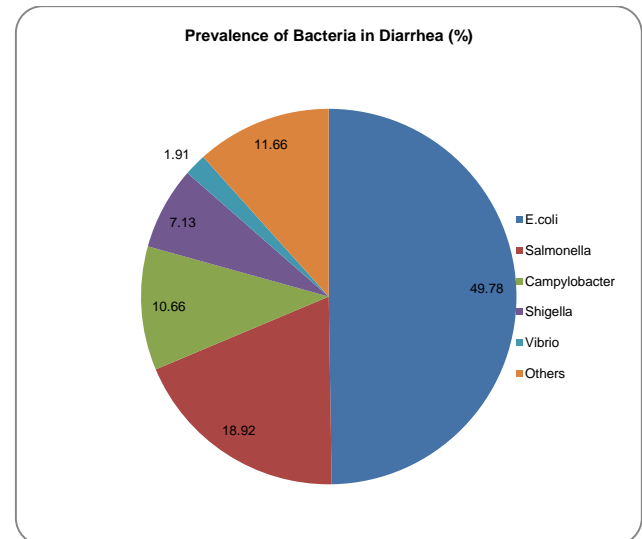


Figure 1: Prevalence of bacterial etiology in acute diarrhea

The age-wise prevalence was recorded and the pediatric patients under 1 year of age were significantly affected ( $p < 0.05$ ) with acute diarrhea caused by bacteria (76/147; 51.70%) and parasites (71/147; 48.29%), followed by subsequent age groups of 1-2 (76/384; 19.79%), 2-3 (65/384; 16.92%), 3-4 (60/384; 15.62%) and 4-5 years (36/384; 9.375%). Their comparative values of

bacterial and parasitic origin were expressed below (Table 2). Sex-wise prevalence of acute diarrhea in children revealed that 51.04% of male children and 48.95% of females were affected with a non-significant difference ( $p \geq 0.05$ ) but the underlying etiology of parasitic origin was statistically significantly higher ( $p < 0.05$ ) than the bacterial origin (Table 3). The children from the rural population were significantly affected by diarrhea with a prevalence rate of 59.11% (227/384) than the urban kids at 40.88% (Table 4).

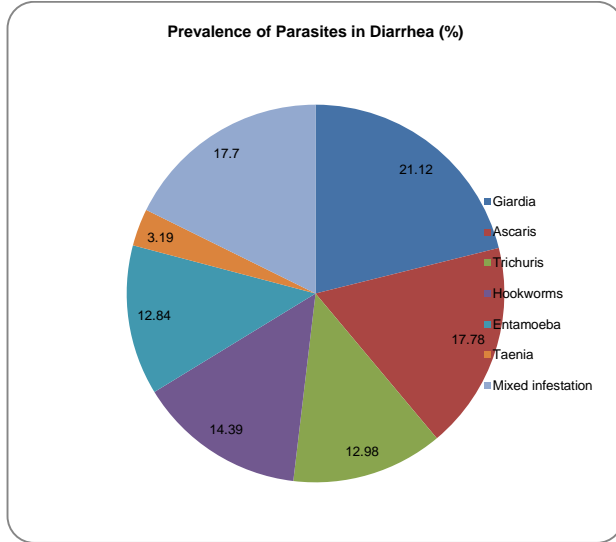


Figure 2: Prevalence of parasitic etiology in acute diarrhea

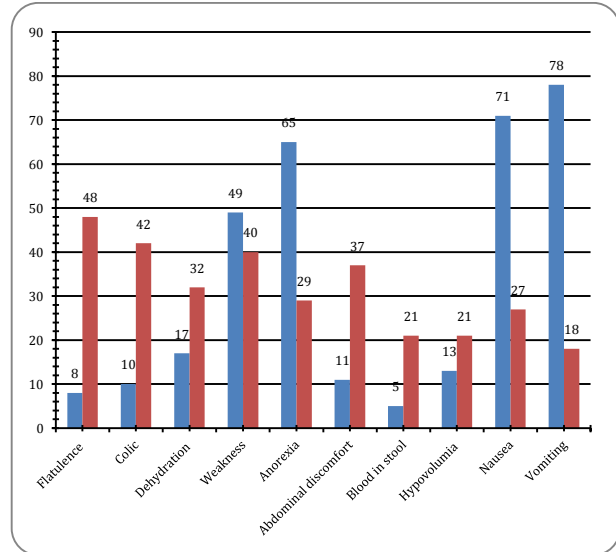


Figure 3: Clinical manifestations of acute diarrhea with bacterial and parasitic etiology

Table 2: Age-wise prevalence of bacterial and parasitic ailments in acute diarrhea

S. No	Age Groups	No. of patients	Bacterial Isolates n(%)	Parasites n(%)	p-value
1	<1 Year	147	76 (51.70)	71 (48.29)	0.05560
2	1-2 Years	76	17 (22.36)	59 (77.63)	0.00014*
3	2-3 Years	65	13 (20.0)	52 (80.0)	0.00010*
4	3-4 Years	60	9 (15.0)	51 (85.0)	0.00001*
5	4-5 Years	36	8 (22.22)	28 (77.77)	0.01041*
Total		384	123	261	0.00001*

\*indicated the value is significant at  $p < 0.05$

Table 3: Sex-wise prevalence of bacterial and parasitic ailments in acute diarrhea

S. No	Sex Groups	No. of patients	Bacterial Isolates n(%)	Parasites n(%)	p-value
1	Males	196	62 (31.63)	134 (68.36)	0.00034*
2	Females	188	61 (32.44)	127 (67.55)	0.00011*

\*indicated the value is significant at  $p < 0.05$

Table 4: Location-wise prevalence of bacterial and parasitic ailments in acute diarrhea

S. No	Location	No. of patients	Bacterial Isolates n(%)	Parasites n(%)	p-value
1	Rural	227	69 (56.09)	158 (60.53)	0.00001*
2	Urban	157	54 (43.90)	103 (39.47)	0.00185*

\*indicated the value is significant at  $p < 0.05$

## DISCUSSION

Our findings revealed that most of the children ( $p < 0.05$ ) were affected with parasitic diarrhea 67.96% than bacterial diarrhea (32.03%). The bacterial diarrhea was mostly caused by *Escherichia coli*, *Salmonella*, *Campylobacter* and *Shigella*, while the parasitic diarrhea was mostly associated with *Giardia*, *Ascaris*, hookworms, *Trichuris*, *Entamoeba* and *Taenia*. The clinical manifestations were characterized by vomiting, nausea, anorexia, weakness, dehydration, flatulence, colic, weakness, dehydration, abdominal discomfort and nausea. The age-wise prevalence revealed that pediatric patients under 1 year of age were mostly affected with acute diarrhea followed by subsequent age groups.

Our results were supported by a study conducted in Lebanon, which reported that globally, acute gastroenteritis is a leading cause of illness and mortality in children. It remains a common reason for admissions to emergency units due to infection in all age groups. And the prevalence of *Entamoeba histolytica* was found (27.8%) in acute gastroenteritis in children<sup>8</sup>. Another study revealed the prevalence of *Shigella* species as (10.7%) across all age groups, while the investigations conducted in Bahir Dar, Ethiopia, revealed a prevalence of 8.4%<sup>4, 11</sup>. Most of the children were affected with parasitic diarrhea, because younger children are exposed to a greater number of environmental factors, such as consuming contaminated food, playing with contaminated soil and consuming contaminated water, than older children. They are immunologically naive, and their delayed humoral immune response makes them more susceptible to infection<sup>4, 12</sup>.

Our findings were also in agreement with the study found that diarrheal ailments were the second leading cause of high morbidity and mortality among children under five and 11.0% of children under the age of five were diagnosed with diarrhea in Indonesia. Among the bacterial causes, Enterotoxigenic *E. coli* was identified as the most prevalent bacteria (9.98%), followed by *Campylobacter jejuni* and *Shigella*<sup>13</sup>.

Another related study identified the enteropathogen causing the disease in 53.6% of the AGE hospitalized pediatric patients, which is fewer than the 67% reported by Valenzuela et al. in Chile in 2018 using the Film Array Gastrointestinal panel diagnostic approach<sup>14</sup>. The prevalence rates of AGE-causing enteropathogens in other Middle Eastern nations were 28% in Bahrain<sup>15</sup>, 63% in Palestine<sup>16</sup>, 53.4% in Saudi Arabia<sup>17</sup>, and 57.4% in Lebanon<sup>18</sup>.

Out of 332 (53.6%) detected pathogen-infected AGE cases, 88.55% ( $n = 294/332$ ) were due to a single pathogen infection while 11.45% ( $n = 38/332$ ) were due to mixed pathogens, which is higher than the number of co-infections obtained by an Italian study in 2018 (2.3%)<sup>8, 17</sup> and much lower than the number of concurrent infections provided by Shrivastava et al. from Odisha<sup>19-20</sup>.

## CONCLUSION

Acute diarrhea is the second leading cause of mortality in children and it infected a significant proportion of children under five years of age. Most of the diarrheic patients were diagnosed with parasitic and bacterial etiological agents. The major risk factors of the infection were poor hygiene, sanitation, weak immunity and playing of children in the contaminated soil and ingesting contaminated

food materials. The disease can easily be prevented in children by improving their living standards, nutrition, sanitation and hygiene.

**Conflict of Interest:** None

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