



# Retrospective evaluation of gastrointestinal system infections: Investigation of viral, bacterial, and parasitic etiological agents

✉ Tugba Kula Atik<sup>a,\*</sup>, ✉ Alev Cetin Duran<sup>b</sup>, ✉ Digdem Ozer Yildirim<sup>c</sup>, ✉ Ali Duran<sup>d</sup>

<sup>a</sup>Balikesir University, Faculty of Medicine, Department of Medical Microbiology, Balikesir, Turkey

<sup>b</sup>Balikesir Atatürk City Hospital, Department of Medical Microbiology-Basic Immunology, Balikesir, Turkey

<sup>c</sup>Balikesir Atatürk City Hospital, Department of Infectious Disease, Balikesir, Turkey.

<sup>d</sup>Balikesir University, Faculty of Medicine, Department of General Surgery, Balikesir, Turkey

## Abstract

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**Aim:** We aimed to retrospectively investigate the viral, bacterial, and parasitic etiological agents detected in patients to present with gastrointestinal complaints and examine their distribution in our region.

**Materials and Methods:** Patients who presented to the XXX Hospital due to gastrointestinal symptoms between January 2017 and December 2019 were included in the study. The results obtained using conventional culture and immunochromatographic (IC) methods from the stool samples of the patients for etiological diagnosis in the microbiology laboratory were retrospectively evaluated. The infectious etiological agents were analyzed according to the age groups. Clinical and epidemiologic characteristics of the agents have been described.

**Results:** The positivity rates were 6.6%, 2.2%, and 0.4% for Rotavirus (RV), Adenovirus (AV), and Norovirus (NV); 0.8%, 2.8%, and 0.4% for *Salmonella* spp., *Helicobacter pylori*, and *Clostridium difficile*; and 2.1% and 1.1% for *Entamoeba histolytica* and *Cryptosporidium* spp., respectively. *Shigella* spp. and *Giardia intestinalis* were not detected in any of the samples. The highest positivity rates in the 0–2, 3–10 and 11–20 age groups were found for RV, whereas in the 21–40, 41–60 and > 60 age groups were determined for *H. pylori*. RV infections were observed predominantly in the spring.

**Conclusion:** IC methods are a helpful tool for the routine diagnosis of gastrointestinal infections at hospitals. The agent with the highest positivity rate was RV. Still, the overall positivity rates were low due to the good infrastructure of our city and the successful execution of sanitation measures.



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## Introduction

Gastrointestinal system infections (GSI) are prominent among infectious diseases that are frequently detected worldwide. Gastrointestinal pathogens comprise a large panel that includes various bacterial, viral, and parasitic agents [1-3]. The causes of GSI can vary according to age, environmental conditions, geographic regions, and seasons [1, 4, 5].

Viral gastroenteritis (GE) is more of a problem for developed countries regarding successful sanitation programs, while bacterial and parasitic agents are more significant in developing countries [4, 6, 7]. Rotavirus (RV) infections, which particularly affect children under two years of age and cause more severe clinical manifestations, are among the most important causes of childhood GE [4, 8-11]. The

World Health Organization recommended the inclusion of the RV vaccine in national immunization programs in 2013 [12]. Although the RV vaccine is not a part of the routine vaccination program in Turkey, it is predicted that vaccination awareness will increase and RV infections will decrease with individual vaccination activities [9, 10]. Just as RV, adenovirus (AV) infections are more common in childhood, but they cause a milder disease than RV [9]. Norovirus (NV) is one of the agents that cause GE in all age groups, including older children and adults [1, 10, 11].

Bacterial GE agents such as *Salmonella* spp. and *Shigella* spp. is observed in our country and globally [13-15]. While GE caused by *Salmonella* spp. is usually self-limiting and resolves within a week, GE caused by *Shigella* spp. typically requires antibiotics for its management [2, 3, 13, 14]. *Helicobacter pylori* is a bacterial agent that causes a wide variety of gastrointestinal problems such as chronic gastritis, peptic ulcer, and stomach cancer [16, 17]. Toxic strains

\*Corresponding author:

Email address: [tkulaatik@gmail.com](mailto:tkulaatik@gmail.com) (✉ Tugba Kula Atik)

of *Clostridium difficile* are the most common causes of GE, especially in hospitalized patients receiving antibiotic therapy [18].

Parasitic diseases of the gastrointestinal tract constitute a major problem worldwide, more so in developing countries [19]. In addition to viral and bacterial agents that can cause GSI, the most frequently detected parasitic agents are *Entamoeba histolytica*, *Giardia intestinalis*, and *Cryptosporidium* spp. However, these agents are sometimes overlooked due to their relatively low incidence [14, 19].

Microscopic examination of stool samples is very important in the microbiological diagnosis of GSI, and culture is generally preferred to evaluate bacterial agents [2, 3, 14]. Different methods such as immunochromatography (IC), latex agglutination, enzyme-linked immunosorbent test (ELISA), and polymerase chain reaction (PCR) can be used as stool antigen screening methods [2, 3, 8, 19]. IC methods, which are easy to use and provide rapid results, are widely used in laboratories to diagnose viral, bacterial, and parasitic agents that can cause GSI [2, 3, 5, 8].

The broad spectrum of GE agents and the inability to identify all agents in routine laboratory conditions result in the excessive use of empirical antibiotics [5, 15]. Therefore, knowing the regional distribution of the agents causing GSI can help determine the correct diagnosis and treatment algorithms and prevent antibiotic abuse [4, 5, 10, 15].

Our study aimed to retrospectively investigate the viral, bacterial, and parasitic etiological agents detected in patients to present with gastrointestinal complaints and examine their distribution in our region, as well as their distribution according to the gender, seasons and hospitalization status of the patients.

## Materials And Methods

### Study design and setting

The local ethics committee approved this study (Decision dated 11.11.2020, decision number 2020/204). Patients who presented to the XXX Hospital due to gastrointestinal symptoms between January 2017 and December 2019 were included in the study. The results obtained using conventional culture and IC methods from the stool samples of the patients for etiological diagnosis in the microbiology laboratory were retrospectively evaluated.

The infectious etiological agents were analyzed according to the age groups (0–2 years, 3–10 years, 11–20 years, 21–40 years, 41–60 years, and > 60 years). In addition, the distribution of positive viral agents was analyzed according to the seasons, gender, and hospitalization status. Data on foreign nationals were excluded from the study.

### Laboratory methods

#### Culture

For the culture of stool samples; 5% sheep blood agar, eosin-methylene blue agar, and Salmonella–Shigella agar were used. Suspected colonies of *Salmonella* spp. and *Shigella* spp. grown in the plates incubated for 24–48 hours at 37°C were identified using conventional methods and BD Phoenix 100 automated identification system (BD Phoenix System, Beckton–Dickinson, USA). The

antibiotic susceptibilities of the isolates were determined according to the European Committee on Antimicrobial Susceptibility Testing criteria using the same automated system (20).

### Rapid stool antigen tests for viruses, bacteria, and parasites

According to the manufacturer's instructions, IC tests were used to detect the viral agents of RV (Rotavirus-Adenovirus Combi Test Kit, Türklab, Turkey), AV (Rotavirus-Adenovirus Combi Test Kit, Türklab, Turkey), and NV (RIDA®QUICK Norovirus, R-Biopharm AG, Germany); bacterial agents of *H. pylori* (Helicobacter pylori Antigen Test Kit, RDS, Turkey) and *C. difficile* (*Clostridium difficile* Toxin A+B, Certest, Spain); and the parasitic agents of *E. histolytica* (*Entamoeba histolytica* Rapid Test Cassette, Acro Biotech, USA), *G. intestinalis* (RIDA®QUICK *Giardia*, R-Biopharm AG, Germany), and *Cryptosporidium* spp. (*Cryptosporidium* Antigen Rapid Test Cassette, Acro Biotech, USA). Their sensitivity and specificity were 99.9% and 98.4% for RV; 99.9% and 99.0% for AV; 92.0% and 98.0% for NV; 94.1% and 100% for *H. pylori*; > 99.0% and > 99.0% for *C. difficile*; 95.7% and 99.2% for *E. histolytica*; 100% and 95.2% for *G. intestinalis*; and 95.2% and 97.8% for *Cryptosporidium* spp., respectively.

### Statistical analysis

The data obtained in the study were recorded in the SPSS 22.0 (SPSS INC, Chicago, IL, USA) program and statistical analysis was made. Categorical variables were given as percentage and mean±standard deviation. Chi-square was used to compare independent groups with categorical variables. The *p* value < 0.05 was considered statistically significant.

## Results

In our study, we found that 351 positivity in 5, 281 patients for RV (6.6%), 115 positivity in 5, 264 patients for AV (2.2%), 14 positivity in 3, 552 patients for NV (0.4%), 36 positivity in 4689 patients for *Salmonella* spp. (0.8%), 63 positivity in 2, 271 patients for *H. pylori* (2.8%), 7 positivity in 1, 708 patients for *C. difficile* (0.4%), 116 positivity in 5612 patients for *E. histolytica* (2.1%), 8 positivity in 696 patients for *Cryptosporidium* spp. (1.1%). *Shigella* spp. was not detected in any of the 4, 689 samples and *G. intestinalis* was not detected in any of the 1319 samples. The agent with the highest positivity rate was RV (6.6%). The coexistence of RV and AV was detected in 31 patients (0.6%), AV and NV in two patients (0.1%), and all three viral agents in one patient (0.02%).

The age-wise distribution of the etiological agents detected in our study is summarized in Table 1. The highest positivity rates in the 0–2, 3–10 and 11–20 age groups were found for RV, whereas in the 21–40, 41–60 and > 60 age groups were determined for *H. pylori*. When the age groups were compared according to the horizontal order in the table, the highest positivity rates were found for RV, *Cryptosporidium* spp. and *C. difficile* in the 0–2 age group; for AV, *Salmonella* spp., and *E. histolytica* in the

**Table 1.** Distribution of the etiological agents based on the age

Pathogens	Age (mean±SD)		Age groups (year)													
			0-2		3-10		11-20		21-40		41-60		> 60			
	Total	+(%)	Total(n)	+(%)	Total (n)	+(%)	Total (n)	+(%)	Total (n)	+(%)	Total (n)	+(%)	Total (n)	+(%)		
<b>Virus</b>																
Rotavirus	10.4±17.9	3.7±8.1	2,551	234 (9.2)	1448	94 (6.5)	477	12 (2.5)	363	7 (1.9)	217	2 (0.9)	225	2 (0.8)	5,281	351 (6.6)
Adenovirus	10.3±17.9	4.8±9.4	2,516	61 (2.4)	1465	43 (2.9)	489	6 (1.2)	360	4 (1.1)	207	0	227	1 (0.4)	5,264	115 (2.2)
Norovirus	11.5±19.1	8.7±15.0	1,642	6 (0.4)	950	6 (0.6)	347	1 (0.3)	272	0	151	1 (0.7)	190	0	3,552	14 (0.4)
<b>Bacteria</b>																
<i>Salmonella</i> spp.	34.7±23.6	32.8±27.5	412	4 (0.9)	464	10 (2.2)	372	4 (1.1)	1753	5 (0.3)	899	5 (0.6)	789	8 (1.0)	4,689	36 (0.8)
<i>Shigella</i> spp.	34.7±23.6		412	0	464	0	372	0	1753	0	899	0	789	0	4,689	0
<i>H. pylori</i>	24.0±23.4	27.0±24.1	509	13 (2.6)	440	13 (3.0)	346	4 (1.2)	354	12 (3.4)	408	15 (3.7)	214	6 (2.8)	2,271	63 (2.8)
<i>C. difficile</i>	27.3±26.6	25.2±30.5	468	4 (0.9)	230	0	148	0	344	0	244	2 (0.8)	274	1 (0.4)	1,708	7 (0.4)
<b>Parasite</b>																
<i>E. histolytica</i>	15.9±21.1	17.3±20.9	2,024	28 (1.4)	1379	41 (3.0)	681	14 (2.1)	722	15 (2.1)	436	10 (2.3)	370	8 (2.2)	5,612	116 (2.1)
<i>G. intestinalis</i>	16.6±21.3		415	0	356	0	185	0	171	0	105	0	87	0	1,319	0
<i>Cryptosporidium</i> spp.	13.7±22.3	2.0±4.5	292	7 (2.4)	206	1 (0.5)	68	0	37	0	39	0	54	0	696	8 (1.1)

\*Positive samples

**Table 2.** Distribution of the viral agents detected positive according to the seasons, gender, and patient’s hospitalization status

	Rotavirus (n:351)	Adenovirus (n:115)	Norovirus (n:14)
<b>Season n (%)</b>			
Spring	187 (53.3)	35 (30.4)	6 (42.9)
Summer	60 (17.1)	32 (27.8)	4 (28.5)
Autumn	34 (9.7)	29 (25.2)	2 (14.3)
Winter	70 (19.9)	19 (16.6)	2 (14.3)
<b>Gender n (%)</b>			
Male	189 (53.8)	62 (53.9)	6 (42.9)
Female	162 (46.2)	53 (46.1)	8 (57.1)
<b>Patient’s hospitalization status n (%)</b>			
Inpatient	104 (29.6)	23 (20.0)	4 (28.6)
Outpatient	247 (70.4)	92 (80.0)	10 (71.4)

3-10 age group; for *H. pylori* and NV in the 41-60 age group. The agent with the highest mean age of the patients was *Salmonella* spp. (mean age: 32.8±27.5), while that with the lowest mean age was *Cryptosporidium* spp. (mean age: 2.0±4.5). The mean age of the patients with coexistence of RV and AV was 4.9±7.9. Resistance of the *Salmonella* spp. isolates to ciprofloxacin, ampicillin, and trimethoprim–sulfamethoxazole (SXT) were 17.9%, 7.2%, and 4.0%, respectively.

The distribution of the detected viral agents according to the seasons, gender, and hospitalization status of the patients is given in Table 2. RV infections were observed predominantly in the spring ( $p < 0.005$ ). RV, AV and NV infections were found to have similar characteristics in terms of distribution according to the gender ( $p < 0.199$ ). That infections were examined in terms of distribution according to the hospitalization status and the highest positivity rates were found in patients who received outpatient services ( $p < 0.279$ ).

**Discussion**

The most common cause of GE is viruses such as RV (the most frequent etiologic agent), AV, and NV. The most common bacterial agents are *Salmonella* spp. and *Campylobacter* spp., and the most common parasitic agents are *G. intestinalis* and *E. histolytica* [15, 21]. RV is one of the 13 etiologic agents that cause GE, as estimated by the 2016 Global Burden of Disease Study. It is the most prevalent agent that causes severe diarrhea in developed and developing countries [22]. In our study too, the highest positivity rate was determined for RV (6.6%), followed by *H. pylori* (2.8%), AV (2.2%), *E. histolytica* (2.1%), *Cryptosporidium* spp. (1.1%), *Salmonella* spp. (0.8%), NV (0.4%), and *C. difficile* (0.4%). González-Serrano et al. [2] found viral (32%), bacterial (14%) and parasitic (1%) agents among the GSI agents. In the study by Oğuztürk et al. [5], only adult patients were included, the most frequently detected microorganisms were protozoans with a detection rate of 54.1%, followed by bacteria with a rate of 27.9% and viruses with a rate of 14.7%.

While PCR is the most sensitive method for detecting viral antigens in stool, IC methods are frequently used in routine laboratory applications [23]. It has been reported that the specificity of IC methods in diagnosis is > 97%, and their sensitivity is > 80% when compared with PCR; furthermore, it is recommended since they are easy to use, low cost, and provide results in a short time [2]. In a meta-analysis study, IC was shown to be the most common method (64.5%) used in the diagnosis of RV in Turkey [8]. In our study, RV positivity was 6.6%, and AV positivity was 2.2%. In studies where IC methods were used, and pediatric and adult patients were collectively evaluated, as in our study, RV positivity rates ranged from 3.6% to 17.3%, and AV positivity rates ranged from 0% to 4% [2, 10, 14, 24].

Our study showed that the mean age of patients with RV positivity was 3.7±8.1 years, and the mean age of patients with AV positivity was 4.8±9.4 years. The highest RV positivity rate was in the 0–2 age group (9.2%), and the AV positivity rate was the highest in the 3-10 age group

(2.9%). Consistent with our work, different studies have emphasized that RV and AV infections affect young children more frequently [2, 4, 8, 14, 15, 24, 25]. In studies where only pediatric patients were evaluated, RV and AV positivity rates were between 9.5%-48.9% and 2.6%-8.6%, respectively [4, 8, 9, 21, 26, 27]. The rates reported in various studies can be affected by different variables such as geographical region, a diagnostic method used, the patient population included in the study, and RV vaccine administrations that have become widespread in recent years [10, 23]. We think that these rates will decrease in the future as all countries worldwide include the RV vaccine application recommended by the World Health Organization in their national immunization programs [12].

RV and AV infections mainly affect children but may also pose a risk for adults [2, 11]. Among adult patients, RV and AV positivity rates were 4.7% and 1.3% [5]. In our study, RV and AV positivity rates were 0.8% and 0.4% for those > 65 years of age. These findings made us think that the investigation of RV and AV should not be limited to pediatric patients.

Studies have shown that NVs are the most common cause of GE, especially in developed countries, and NV positivity rates have increased with the widespread use of RV vaccines [24, 28]. While some sources have reported NV as the second most common agent after RV in acute GE, they have emphasized that it had a much milder course than RV [24, 26]. In our study, the rate of NV positivity was 0.4%. The rates of NV positivity detected using IC, ELISA, or PCR methods vary between 4% and 24% in studies where pediatric and adult patients were evaluated together, as in our study [1, 2, 11, 24]. In our study, NV positivity rates detected using IC methods were far below the literature data. A study comparing IC and PCR methods in the diagnosis of NV showed that the specificity of IC tests was > 97%, and the sensitivity was 57%. While IC techniques are preferred for the diagnosis of viral antigens due to their ease of application, their low sensitivity for NV reveals that the technique should be improved in terms of the diagnosis of NV [2]. We think that the positivity rate observed in our study could have been lower than that reported by other studies because we employed only IC methods.

Several etiological factors may coexist in GE [4, 9, 10]. Various studies have shown that the rates of RV and AV association vary between 0.3% and 8.1% [4, 9, 10, 21]. Similar to these rates, the rate of RV and AV association was 0.6% in our study. Moreover, the rate of AV and NV association was 0.1%. Correspondingly, the rate of AV and NV association was 0.3% in another study [21]. It has been reported that the mixed viral infection positivity rate in stool samples of hospitalized children with acute GE was 3.2% [26]. In our study, the association rate of all three viral agents was determined to be 0.02%.

The most common bacterial agents causing GE include *Campylobacter* spp., *Shigella* spp., and *Salmonella* spp. [14]. In our study, while the *Salmonella* spp. isolation rate was 0.8%, there was no *Shigella* spp. positivity. Similarly, different studies have reported that *Salmonella* spp. isolation rates from stool cultures vary between 0.0% and 6.7%, while *Shigella* spp. isolation rates vary between

0.0% and 3.3% [2, 5, 13-15]. In our study, the highest *Salmonella* spp. positivity rate was in the 3-10 age group (2.2%). Similarly, another study has demonstrated that the isolation rate of *Salmonella* spp. in children ≤5 years of age is 3% [15]. Our study showed that ciprofloxacin resistance was 17.9%, ampicillin resistance was 7.2%, and SXT resistance was 4.0% in *Salmonella* spp. isolates. In another study, SXT resistance was 29%, and resistance to ciprofloxacin, levofloxacin, and ampicillin was not detected [13]. In another study, ampicillin resistance was 53.3%, SXT resistance was 33.3%, and ciprofloxacin resistance was not observed [5]. Considering that antimicrobial resistance rates can vary between centers, antibiotic sensitivity results are beneficial in directing the treatment for GE.

In our study, *H. pylori* positivity was 2.8% (mean age: 27.0±24.1), and the highest rate (3.7%) was found in the 41-60 age group. In another study, it has been reported that the highest prevalence of *H. pylori* occurred in the 30-39 age group [16]. *C. difficile* Toxin AB positivity rate was 0.4% in our study; however, the rate of Toxin AB positivity was 1.3% in another study [5].

The most common parasitic agents in gastrointestinal infections are *E. histolytica*, *G. intestinalis*, and *Cryptosporidium* spp. [14]. Numerous immunoserological methods are used for the detection of parasitic agents in stool samples. These methods have higher sensitivity compared to stool microscopy, while their specificity and cost are relatively comparable [23]. The rate of *E. histolytica* positivity was found to be 2.1% in our study. Various studies have indicated that *E. histolytica* positivity rates vary between 6.0% and 21.8% [5, 14]. We think that these differences may be due to both methodological and geographical regional differences in the patient groups included in the study. The mean age of patients with *E. histolytica* positivity in our study was 17.3±20.9 years, and different studies have emphasized that *E. histolytica* positivity is higher in adult patients [3, 14]. The *Cryptosporidium* spp. positivity rate was 1.1% in our study, and the positivity rates ranged from 1.0%-1.3% in different studies [2, 5].

RV infections are mostly seen in the winter months [4, 9, 10, 27]. On the other hand, our study showed that RV infections were predominantly observed in the spring months ( $p < 0.005$ ). Various studies have emphasized that RV infections are seen at high rates in the winter and spring months [11, 21, 28]. In our study RV, AV and NV infections were found to have similar characteristics in terms of distribution according to the gender ( $p < 0.199$ ) likely as reported in the literature [4, 9, 10, 21]. The highest positivity rates of the RV, AV, and NV infections were found in patients who received outpatient services in our study ( $p < 0.279$ ). With the widespread use of RV vaccines, a significant decrease has been observed in hospitalizations due to RV infection [10, 29].

Our study, in which we retrospectively analyzed viral, bacterial, and parasitic infectious agents detected in patients who applied to our hospital with gastrointestinal complaints, has some limitations. The inability to analyze stool microscopy results retrospectively due to technical inadequacies, the inability to study *Campylobacter* spp., and the failure to confirm stool antigen tests with ELISA



and molecular methods are among these limitations. The infectious etiologic agent with the highest positivity rate was RV, but the overall positivity rates were low. We think that these low positivity rates may be due to the good infrastructure of our city and the successful execution of sanitation measures.

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