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DETERMINATION OF THE ETIOLOGICAL STRUCTURE OF ACUTE INTESTINAL INFECTIONS IN CHILDREN

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Abstract
Background: The high incidence of acute intestinal infections (AII) among children, second only to respiratory pathology, the frequency of adverse disease outcomes, and the low level of laboratory diagnosis have highlighted the medical and social significance of research aimed at studying this problem.
Aim: To provide a clinical-laboratory characterization of acute intestinal infections in children based on the etiological factor.
Methods: The study involved 160 children hospitalized in the departments of the Republican Specialized Scientific-Practical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases in 2023 with a diagnosis of AII. Bacteriological methods and fecal PCR screening were used for etiological diagnosis. The material for bacteriological analysis was the stools of the examined patients, which were tested using the serial dilution method according to Epstein-Litvak (1977) with additions by N.M. Gracheva (1987) and modified by A.M.-T. Bektemirov (1992). For molecular-genetic identification of the pathogen, PCR diagnostic kits from Inter Lab Service "Amplisens® AII Screen-FL" were used to detect and differentiate DNA of microorganisms from the genera Shigella (Shigella spp.), Salmonella (Salmonella spp.), thermophilic Campylobacter (Campylobacter spp.), adenoviruses group F (Adenovirus F), RNA rotaviruses group A (Rotavirus A), noroviruses genotype 2 (Norovirus genotype 2), and astroviruses (Astrovirus).
Results: The etiological factor was identified in 103 (64.38%) cases, while 54 (33.75%) patients had no identified etiological factor. The patients with established etiological diagnoses were divided into three groups: Group 1 consisted of 26 patients with bacterial infections (BAI), Group 2 comprised 45 patients with viral AII (VAI), and Group 3 included 32 patients with viral-bacterial associations (VBAI). In the BAI group, 69.2% of the cases were found in patients under 4 years old, while the VAI group consisted of 95.6% children under 3 years old. In the VBAI group, the disease was also predominantly (28; 87.5%) found in children under 3 years old. The etiological structure of AII showed a significantly higher number of viral diarrhea cases compared to bacterial AII, with a high rate of AII of unidentified etiology.
Conclusion: The practical feasibility of comprehensive fecal testing for children with AII, including both bacteriological and PCR diagnostic methods, has been established. This approach significantly improves the effectiveness of etiological diagnosis by identifying viral and mixed AII in young children, the number of which has recently increased.

Keywords: Acute intestinal infections, viral AII, bacterial AII, viral-bacterial AII, etiological factor

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Overview. Acute Intestinal Infection (AII) is one of the most common infectious diseases, the eighth leading cause of death in all age groups and the fifth leading cause of death in children under five years of age worldwide. It affects between 3 and 5 million children each year, killing nearly 446,000. International migration, the interstate exchange of food and raw materials of animal origin, the intensification of industrial production of livestock and poultry products, urbanisation, the intensification of leisure activities, climate change and environmental degradation are all contributing factors. Viruses are the dominant aetiological factors both in the seasonal increase in AKI incidence (65-76% of cases) and in the analysis of sporadic cases (62.6%). The most common and significant viral pathogens of AKI are rotaviruses, calceoviruses, adenoviruses and astroviruses. For most acute respiratory infections, the immune response developed in childhood provides adequate protection against infection in adulthood. The main reason for the absence or insufficiency of immune defence is the considerable genetic and antigenic diversity of viral pathogens. Molecular genetic methods are the only means of identifying viruses (1,2,3).
Therefore, in order to improve the effectiveness of diagnosis and treatment of intestinal infections, it seems necessary to study the importance of viruses in the aetiological structure of AKI in children using molecular genetic methods, as well as to determine the clinical, immunological, and nitroxidergic aspects of mono- and combined intestinal infections, and to identify risk groups for unfavourable course and development of complications.

Objective: To determine the aetiological structure of acute intestinal infections in children.

Materials And Methods The study included 160 children admitted to the wards of the RSNPCEMIPZ in 2023 with a diagnosis of acute intestinal infection. For the aetiological diagnosis of the disease, a bacteriological method and screening of faeces by PCR were performed.
The material for bacteriological examination was faeces of the examined patients. The bacteriological method of pathogen identification was carried out by serial dilutions according to Epstein-Litvak (1977) with additions by N.M. Gracheva (1987), modified by A.M.-T. Bektimirov (1992), by serial dilution of the examined material with subsequent sowing on dense nutrient media by sectors.
For molecular genetic identification of the pathogen, PCR diagnostic kits of Inter Lab Service company 'AmpliSens®OKI screen-FL' were used for detection and differentiation of DNA of microorganisms of Shigella spp. and Salmonella spp. and thermophilic Campylobacter spp., adenoviruses of group F (adenovirus F) and rotavirus RNA.
Study design The results are from an observational, single-centre, prospective, continuous, uncontrolled study of 160 children admitted to the RSNPCSEMIF wards with a diagnosis of AKI in 2023.

Results.
Of the 160 patients with AKI, diagnosed on the basis of anamnesis, active detection of symptoms, clinical and laboratory tests, 43 were confirmed bacteriologically and 70 were confirmed molecularly and genetically (PCR method).
On the basis of the studies carried out, the following aetiological diagnoses were made AKI of viral etiology (n=45; 28.12%), bacterial (26; 16.25%), combined bacterial-viral intestinal infections (32; 20.0%).

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The criteria for inclusion of patients in the study to determine the aetiological structure of acute intestinal infections in children were: presence of informed consent signed by one of the parents on admission to the hospital; absence of concomitant chronic infectious pathology (viral hepatitis, HIV infection, etc.) and severe concomitant pathology (malformations, cerebral palsy, diabetes mellitus, heart failure, etc.); refusal to sign informed consent; seeking medical help at a late stage in the course of treatment.

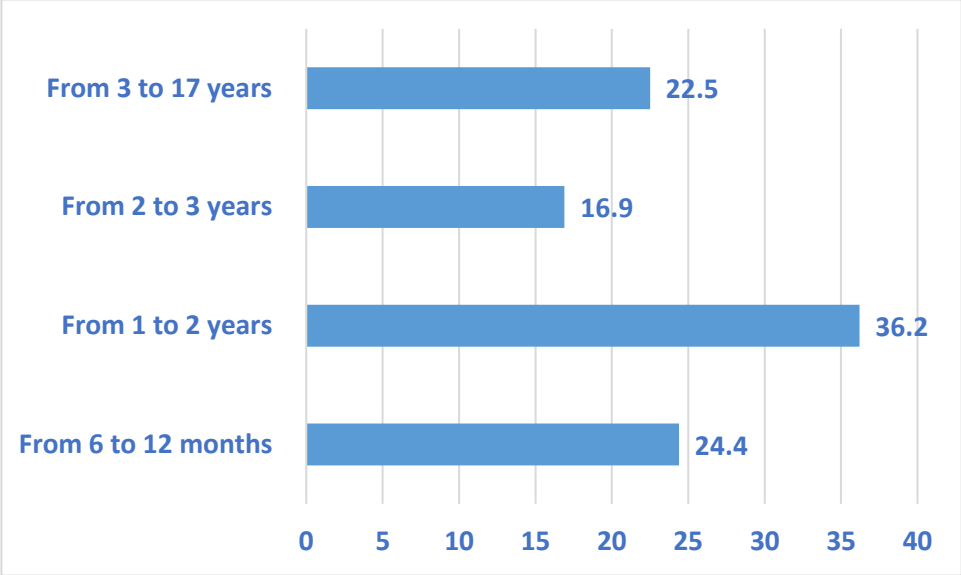

Figure 2. Age structure of patients examined

Thus, more than half of the children (75.0%) were admitted to hospital during the first week of illness, which allowed the study to be conducted and treatment to be started at an early stage of the disease.

In our observations, the mean age of the children was 2.59±0.25 years, with a predominance of boys.

According to the results obtained by the bacteriological method using CXC, the pathogen was identified in 27 (16.9%) cases, of which S. enteriditis was detected in 22 (81.5%) patients, of which it was confirmed by PCR in 6 patients, but in association with Campylobacter spp. in 4 cases, Campylobacter spp.+ rotavirus in 1 case and viral infections (Rota-+Astro-+Norovirus) in 1 patient.

The results of studies on the detection of different groups of intestinal pathogens in stool samples from patients diagnosed with AKI according to the results obtained using the PCR kit 'AmpliSense®OKI Screen-FL' are presented in Table X. In total, positive results were detected in 51.9% of cases (83 samples).9% of cases (83 samples) (Table X), of which monopathogens were detected in 26 cases (31.3%), including: bacterial - 7.7% (Shigella spp. - 3.8%, Campylobacter spp. - 3.8%); viral - 92.3% of cases (Rotavirus - 46.2%, Adenovirus - 23.1%, Astrovirus and Norovirus 2 - 11.5% each).

Bacterio-bacterial associations were detected in 6 cases (7.1%), represented by Salmonella spp. + Shigella spp. - 1.2%, Salmonella spp. + Campylobacter spp. - 5.9%. Viral-bacterial

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associations were represented by associations of 2, 3 and 4 pathogens in 30 (35.3%) cases. Associations of 2 pathogens were detected in 10 cases (11.8%), represented by combinations of Shigella spp. + rotavirus - 3.5%, Shigella spp. + astrovirus - 4.7%, 1 (1.2%) case each of Campylobacter spp. + astrovirus;

When studying the duration and conditions of hospitalisation of the patients, it was found that 10 (38.5%) patients with bacterial AKI (AKI), 9 (20.0%) with viral AKI (VBI) and 4 (12.5%) with viral-bacterial AKI (VBI) were admitted on the first day after the onset of the illness; 46.2% of patients with AKI, 11.1% with VBI and 25% with VBI were admitted on the second day; 15.4%, 68.9% and 62.5% of patients, respectively, were admitted later. Comparative analysis shows that patients with viral AKI were predominantly admitted later (4 days more).

We made a comparative analysis of the gender differences in the groups studied. In the group of patients with bacterial infections, there were 1.25 (57.7%) times more boys, in the group with viral infections - 2 times more (66.7%), in the group with mixed infections - 1.29 times more (56.2%). In other words, the gender of patients with mono-ethiological and combined infections was not significantly different (P>0.05), and there were more boys than girls in all groups.

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Table 1. The specific structure of the aetiological agents of OCI according to the results of a PCR study based on the 'AmpliSens®OCI screen-FL kit'.

№	positive sample variants	total	%
1	*Shigella spp.*	1	1,25
2	*Campylobacter spp.*	1	0,62
3	*Rotavirus*	12	7,5
4	*Norovirus 2*	3	1,9
5	*Adenovirus*	6	3,8
6	*Astrovirus*	3	1,9
	Всего монопатогены	26	16,9
1	*Shigella spp.+ Campylobacter spp.*	1	0,6
2	*Shigella spp.+Astrovirus*	4	2,5
3	*Shigella spp.+Rotovirus*	3	1,9
4	*Salmonella spp..+Campylobacter spp.*	5	3,1
5	*Campylobacter spp.+Rotovirus*	1	0,62
6	*Campylobacter spp.+Norovirus*	1	0,62
7	*Campylobacter spp.+Astrovirus*	1	0,62
8	*Adenovirus+Rotovirus*	4	2,5
9	*Adenovirus+Norovirus*	6	3,8
10	*Astrovirus+Norovirus*	1	0,62
11	*Rotovirus+Astrovirus*	3	1,9
12	*Rotovirus+Norovirus*	2	1,25
		32	20,0
1	*Salmonella spp..+Campylobacter spp.+Rotovirus*	1	0,62
2	*Salmonella spp.+ Shigella spp.+ Adenovirus*	1	0,62
3	*Salmonella spp.+ +Rotovirus+Astrovirus*	2	1,25
4	*Salmonella spp.+ Adenovirus+ Norovirus*	1	0,62
5	*Campylobacter spp. +Rotovirus+Astrovirus*	3	1,9
6	*Campylabacter spp.+ Rotovirus+Adenovirus*	4	2,5
7	*Campylabacter spp.+ Rotovirus+Norovirus*	4	2,5
8	*Campylabacter spp.+ Adenovirus.+Norovirus*	1	0,62
9	*Shigella spp. +Norovirus+Astrovirus*	1	0,62
10	*Shigella spp. +Astrovirus +Adenovirus*	1	0,62

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11	*Rotovirus+Norovirus+Astrovirus*	4	2,5
12	*Rotovirus+Adenovirus+Norovirus*	1	0,62
		24	15,6
1	Salmonella spp.+ Rotovirus+Norovirus+Astrovirus	1	1,8
	Total associations	57	36,2
	total positives	85	53,1

We also carried out a comparative analysis of age-related characteristics. During the course of the study, it became clear that the majority of patients were children under the age of 3.

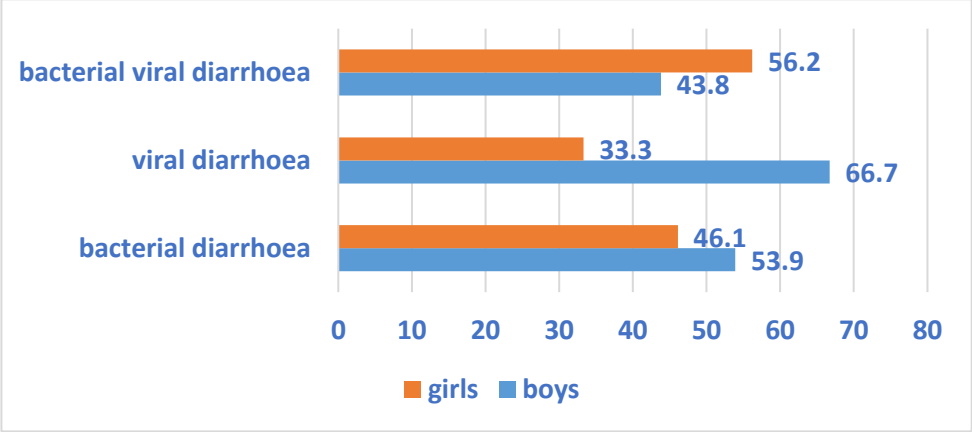

Fig. 1 Distribution of patients examined by sex according to the aetiological structure of diarrhoea (abs.)

In the bacterial AKI group, 69.2% of cases were detected in children under 4 years of age (1 case), whereas in the viral AKI group, only 1 case was detected in a child under 6 years of age (1 case), and 95.6% of cases were in children under 3 years of age. In combined bacterial-viral AKI, the disease was also most commonly diagnosed in children under 3 years of age (28;87.5%).

In the age structure of combined bacterial-viral AKI, viral-bacterial infections (RVI+salmonellosis, campylobacter, shigella - 53.8% of patients) were diagnosed significantly more often in children aged 1 to 3 years than viral-viral infections (RVI+noroviruses, adenrviruses, astroviruses - 26.7% of children, P<0.05). The data obtained should alert the clinician to diagnose and treat this group of patients with the expectation of combined AKI.

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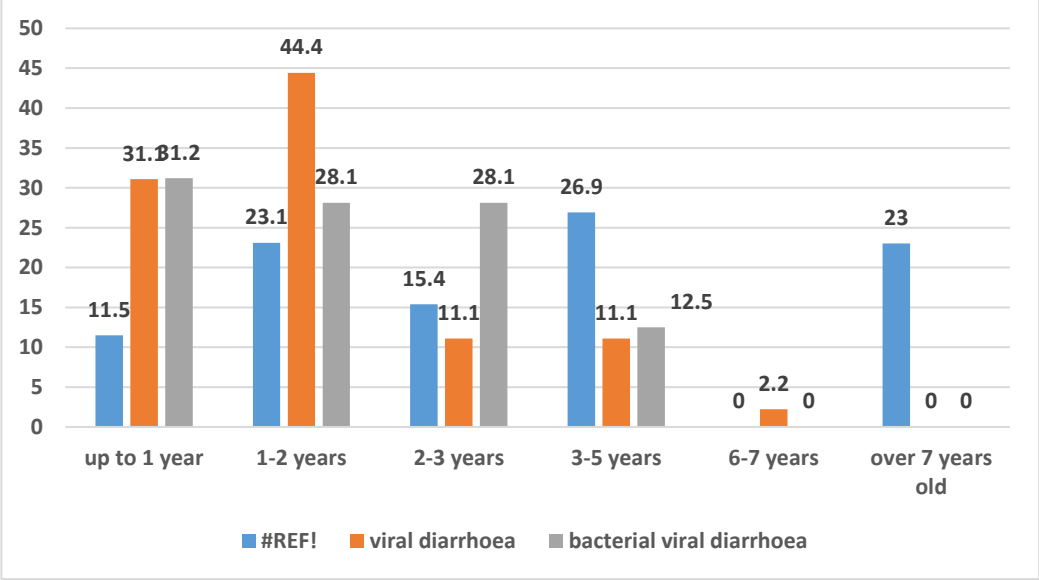

Fig. 2 Age distribution of the examined patients depending on the etiological structure of diarrhoea (abs.)

Analysis of seasonal patterns showed that while bacterial acute intestinal infections (AII) were characterised by an increase in the spring-summer period (May-July), viral and combined AII showed isolated cases in the winter-spring period, with the main increase occurring in the summer months (June, July and August) of the year (36;80.0% and 25;78.1%, respectively).

Among the aetiological factors of acute intestinal infections (AII) in children, viruses now play a prominent role, with rotaviruses and noroviruses being the most important [4,5], while an increase in the proportion of combined forms has been noted [5,6,7]. Our studies show that the aetiological factor in patients with AII was determined by PCR and bacteriological methods in 103 (64.38%) cases, while in 54 (33.75%) patients with AII no aetiological factor was identified. Of all the patients studied with an established aetiological diagnosis by bacteriological and PCR testing, 26 patients were found to have bacterial infections, 45 patients had diarrhoea of viral etiology, and 32 patients had viral-bacterial associations of pathogens. In the bacterial AII group, the disease was detected in children under 4 years of age in 69.2% of cases, while in the viral AII group, 95.6% of patients were children in the first 3 years of life. In combined bacterial-viral AII, the disease was also mainly detected in children under 3 years of age (28; 87.5%).

Conclusions

Our research shows that in the aetiological structure of acute intestinal infections (AII) with established etiology, the number of cases of viral diarrhoea is significantly higher than that of bacterial AII, while the incidence of AII of unknown etiology remains high. The practical feasibility of a comprehensive study of faecal samples from children with AII, including both bacteriological and PCR diagnostic methods, has been demonstrated. This approach allows a significant increase in the effectiveness of aetiological identification, including the detection of viral and mixed AII in young children, the number of which has recently increased significantly.

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