MEDICAL UNIVERSITY OF SOFIA

MEDICAL FACULTY

DEPARTMENT OF INFECTIOUS DISEASES, PARASITOLOGY AND TROPICAL MEDICINE

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CLINICAL-DIAGNOSTIC STUDIES ON VIRAL GASTROENTERTIS IN CHILDREN FROM 0 TO 10 YEARS OF AGE

ABSTRACT

of DISSERTATION PAPER

for the acquisition of the ONS "Doctor" in the field of higher education

7. "Health and sports" by professional direction

7.1. "Medicine" scientific specialty "Infectious diseases"

Supervisor

Associate Professor Nina Yancheva, Ph.D

Sofia, 2023

The dissertation contains **160** typewritten pages and is illustrated with **34** tables and **44** figures. The book collection includes **205** literary sources, of which **21** are in Cyrillic and **184** in Latin.

The numbering of the figures and tables in the abstract is adapted according to the volume of information included and is different from that in the dissertation.

The dissertation was discussed and referred for public defense by the Departmental Council of the Department of Infectious Diseases, Parasitology and Tropical Medicine at the Medical University - Sofia on **July 11, 2023.**

The official defense of the dissertation will take place on **23.11.2023 at 14:00** at the Department of Infectious Diseases, Parasitology and Tropical Medicine of the Faculty of Medicine of the Medical University of Sofia (the auditorium of SBALIPB "Prof. Ivan Kirov" EAD) before a scientific jury in composition:

The dissertation contains 168 typewritten pages and is illustrated with 89 tables and 44 figures. The book collection includes 200 literary sources, of which 20 are in Cyrillic and 180 in Latin. The numbering of the figures and tables in the abstract is adapted according to the volume of information included and is different from that in the dissertation. The dissertation was discussed and referred for public defense by the Departmental Council of the Department of Infectious Diseases, Parasitology and Tropical Medicine at the Medical University - Sofia on July 11, 2023. The official defense of the dissertation will take place on 23.11.2023 at 14:00 at the Department of Infectious Diseases, Parasitology and Tropical Medicine of the Faculty of Medicine of the Medical University of Sofia (the auditorium of SBALIPB "Prof. Ivan Kirov" EAD) before a scientific jury in composition:

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The materials for the defense of the dissertation are published on the website of the Sofia University and are available to those interested in the Secretariat of the Department of Infectious Diseases, Parasitology and Tropical Medicine, "**Acad. Ivan Geshov" No. 17, Sofia.**

CONTENTS:

Abbreviations used:

AGE - adenoviral gastroenteritis

EM - electron microscopy

EU - European Union

IHT-immunochromatographic tests

DNA - deoxyribonucleic acid

MBAL - General Hospital for Active Treatment

NGE - norovirus gastroenteritis

RGE - rotavirus gastroenteritis

RNA-ribonucleic acid

USA - United States of America

SBALIPB-Specialized hospital for active treatment of infectious and parasitic diseases

UMBAL-University General Hospital for Active Treatment

Av-astrovirus

Adv- adenovirus

2A, 2B, 2C, 3A, 3B, 3C - non-structural proteins

HdAv-human adenovirus

B-E- antigenic rotavirus groups

CDC - Center for Communicable Disease Control

DLP - two-layer particle with chelating agents

dntP-deoxyribonucleotide triphosphatase

ECHO-echoviruses

EIA - solid phase microtiter immunoassay

EPA-US Environmental Protection Agency

FC-free chlorine

G1P, G2P, G3P, G4P, -rotavirus genotypes

LIC - small viral peptide of the capsid protein

nm - nanometers

P1, P2, P3-coding regions

Ph - indicator of the acid-base state

RIA-radioimmune enzyme assay

RV-rotavirus

RV1-Rotarix, a human rotavirus strain vaccine

RV5-Rotatec, a reassortant pentavalent vaccine containing human-bovine rotavirus

RT-PCR-Real Time Polymerase Reaction

SRSVs-small round structural viruses

VP1,VP2, VP3,VP4, VP5, VP6, VP7, -glycoproteins, structural components of rotaviruses

Introduction:

Viral intestinal infections are a serious health problem not only in developing but also in developed countries. Until the 1970s, these infections were unknown to mankind, but a high percentage of diarrheal diseases were thought to be caused by them. Since the discovery of noroviruses using electron microscopy, knowledge about them and about other viral enteric infections has continued to grow.

A relatively small number of authors in the world literature compare the demographic and clinical-laboratory characteristics of patients with viral intestinal infections. Even less frequently, similarities and differences in clinical course and length of hospital stay have been described and comparative analyzes have been made between molecular biological methods (RT-PCR) and immunochromatographic tests (ICT) as methods for proving an etiological viral enteric agent in faecal samples. Modern Real Time RT-PCR is increasingly used in clinical practice.

They make it possible to make a relatively quick etiological diagnosis through the use of a method with high sensitivity and specificity. Immunochromatographic tests (IHT) are even faster with the possibility of making the diagnosis at the patient's bedside, which enable the result to be obtained within the fifteenth minute.

In our study, demographic, clinical and paraclinical features were evaluated and compared in children with viral enteric infections aged 0 to 10 years.

I. GOAL AND OBJECTIVES:

Objective: The aim of the present study is to determine the etiological variety of viruses in children with acute diarrhea up to 10 years old, to describe the demographic and clinical-laboratory characteristics of children with viral gastroenteritis, as well as to evaluate the severity of the course and the prognosis of the diseases .

Tasks:

1. To carry out etiological confirmation of rotavirus gastroenteritis
2. To carry out etiological confirmation of adenoviral gastroenteritis
3. To carry out etiological confirmation of norovirus gastroenteritis
4. To carry out etiological confirmation of astrovirus gastroenteritis
5. Comparative analysis of the clinical course of rota-, noro- and viral intestinal unspecified infections
6. Comparative analysis of diagnostic methods - IHT and Real-time PCR in acute viral gastroenteritis

II. MATERIALS AND METHODS:

1. Materials

200 fecal samples of children aged 0 months to 10 years, treated in the SHATIPD" Prof. Ivan Kirov" EAD and Department of Infectious Diseases of MHAL" St. Ivan Rilski" EOOD, town of Dupnitsa for the period January 2018 to December 2022. Their age and gender distribution is presented in Fig.1.

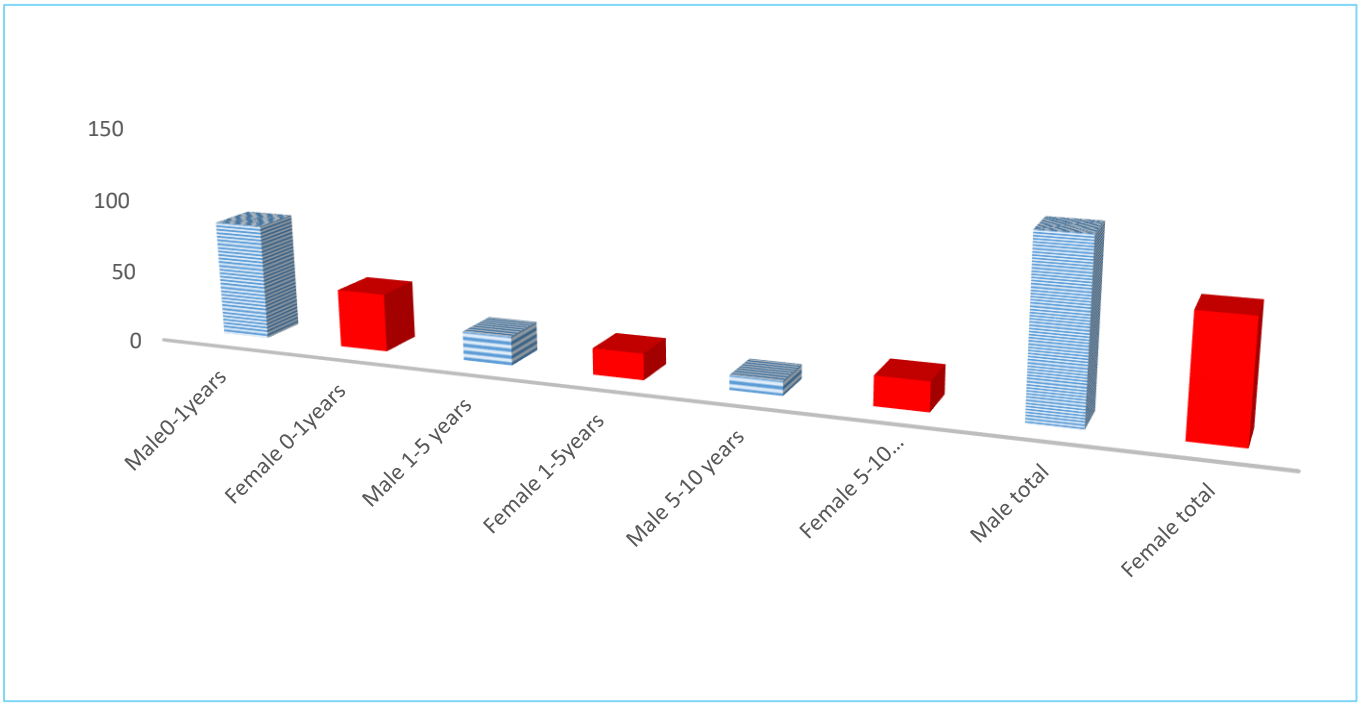

Fig.1. Distribution of participants by gender and age groups

2. Methods

Classical clinical and laboratory methods were used for all patients, and statistical methods were used to process the obtained results

2.1. Clinical methods

The severity of the disease is assessed according to the following criteria:

fever, abdominal pain, nausea, vomiting, diarrhea, respiratory symptoms, and seizure symptoms.

2.1. Laboratory methods

2.2.1 Paraclinical methods

All patients with viral intestinal infections underwent a laboratory analysis of the main indicators of the complete blood count - leukocytes, hemoglobin, platelets and ESR (Clinical Laboratory of SBALIPB "Prof. Ivan Kirov" EAD, headed by Dr. Ivanka Gaberska and clinical laboratory of MBAL "St. Ivan Rilski" EOOD, town of Dupnitsa, headed by Dr. Nedyalko Kalchev).

2.2.2 Specific methods

Immunochromatographic tests were performed on all patients as a rapid test "at the patient's bedside", and the data obtained from them were compared with the data from the molecular biological analysis - Real Time PCR

The PCR-methodology was carried out in the "Enteroviruses" laboratory at the National Center for Disease Control and Prevention, Sofia, under the supervision of head.

Immunochromatographic tests

The immunochromatographic test is the most common test in clinical laboratories worldwide in the field of rapid diagnostics. Rapid tests, also known as immunochromatographic assays, are simple test devices designed to detect the presence (or absence) of a target antigen or antibody in a biological sample (blood, feces, urine, saliva, etc.) without the need for specialized and expensive equipment. The principle of the immunochromatographic test is the same as the ELISA method. The only difference is that the capillary action performs an immunological reaction on the chromatography paper. Two specific antibodies against the antigen are used for this system. One of the antibodies is immobilized on the chromatography paper, while another is labeled with colloidal gold and infiltrated into a sample pad. The immunochromatography module is completed by attaching the sample pad to the end of the membrane. The liquid sample is dropped onto the sample pad. The antigen in the sample forms an immunocomplex with the antibody labeled with colloidal gold. Its complex moves along with the liquid sample and makes contact with the antibody immobilized on the membrane. This is followed by the formation of an immunocomplex with the immobilized antibody. This results in the generation of a colored red-pink line. The appearance of a red-pink line on the membrane indicates the presence of the desired antigen in the sample. The sample liquid migrates through the membrane very quickly, making it possible to detect the presence or absence of antigen within 15 minutes, as shown in the image below.

Components of the immunochromatographic test include:

1.**Sample pad:** acts as a sponge and holds excess liquid from the sample. Once soaked, the liquid migrates to the second element.

2. **Conjugated support**: A dried format of bioactive particles in a matrix that contains everything necessary to ensure an optimized chemical reaction between the target molecule e.g. antigen - antibody that is immobilized on the surface of the particle.

3.**Control:** Contains an antibody that captures free latex/gold to confirm that the test has worked correctly.

4.**Test**: Contains a specific capture molecule and captures only those particles on which an antigen or antibody molecule is immobilized.

When testing a stool sample for antibodies or antigens, a small amount of the sample needs to be mixed with the buffer accompanying the test. Mix well by shaking or vortexing for a few seconds. After that, a few drops are dripped into the well marked for the cell on the test plate. Counts after the fifteenth minute. The manufacturer's instructions are followed for each individual test.

Interpretation of the results of the immunochromatographic test is carried out in the following way:

Negative test: Appearance of only one band in the control area.

Positive test: Appearance of both bands in the test and control regions.

Invalid test: No band at all or band only in the test region.

Note: The test is positive even with a very faint band in the test region.

Immunochromatographic tests are a promising tool for a reliable level of diagnostic performance. Rapid analyzes can be performed in 15-20 minutes. They reduce the need for staff training and expensive equipment. It can be used in harsh field conditions. Unfortunately, the assays are not as sensitive as other immunoassays such as ELISA or RIA (radioimmunoassay). Immunochromatographic test results should be confirmed by other reference tests.

The sensitivity of IHT is 75.8% and the sensitivity -99.2%.

Real Time PCR

Polymerase Chain Reaction PCR is an in vitro technique for multiplying specific DNA regions. It consists of stages: 1. The following Extraction (isolation) of the viral nucleic acid; 2. In the case of viruses with an RNA genome - reverse transcription (Reverse Transcription) - synthesis of complementary DNA (kDNA, cDNA); 3. Amplification of cDNA using virus-specific oligonucleotides (primers) and heat-stable DNA polymerase. The enzyme has the ability to read the original in the 3'-5' direction and synthesize the new complementary DNA strand in the 5'-3' direction using free dinucleotide triphosphatases (dNTPs) as building blocks. 4. Visualization of PCR products (amplicons).

In Real Time PCR, in addition to primers, the reaction mixture also includes a probe (TaqMan® probe) - a specific oligonucleotide that binds to the amplified DNA fragments between the binding sites of the primers. A reporter (a fluorophore emitting fluorescent light) is attached to the 5' end of the probe, and a quencher (a substance that absorbs the fluorescent light of the reporter when the reporter and the quencher are in close proximity) is attached to the 3' end. In the process of building the new complementary DNA molecule, the polymerase degrades the probe, separating the reporter and the quencher. The fluorescent light of the reporter is not absorbed by the quencher, but is emitted, and the radiation is monitored by the PCR detector during each run. Amplification of the target molecule is recorded as the increase with time of fluorescence compared to the background signal. Released reporter molecules accumulate as the number of cycles increases, and thus the increase in fluorescence is proportional to the amount of amplified product.

The Real Time PCR method allows proof, typing and subtyping of enteric viruses and clinical samples and is distinguished by high sensitivity -88% and specificity -99%.

All patients included in the study underwent microbiological tests, carried out by Dr. Ivelina Tomova, head of the microbiological laboratory of SBALIPB Prof. Ivan Kirov EAD, and all results were negative

for bacterial growth. (Fig. 2) Patients whose fecal samples were found to contain bacteria were excluded from the analyzes (Fig. 3).

Parasitological studies were also carried out by Dr. Rositsa Chipeva in the parasitological laboratory of SHATIPB Prof. Ivan Kirov EAD - no helminths and protozoa were found in the examined fecal samples.

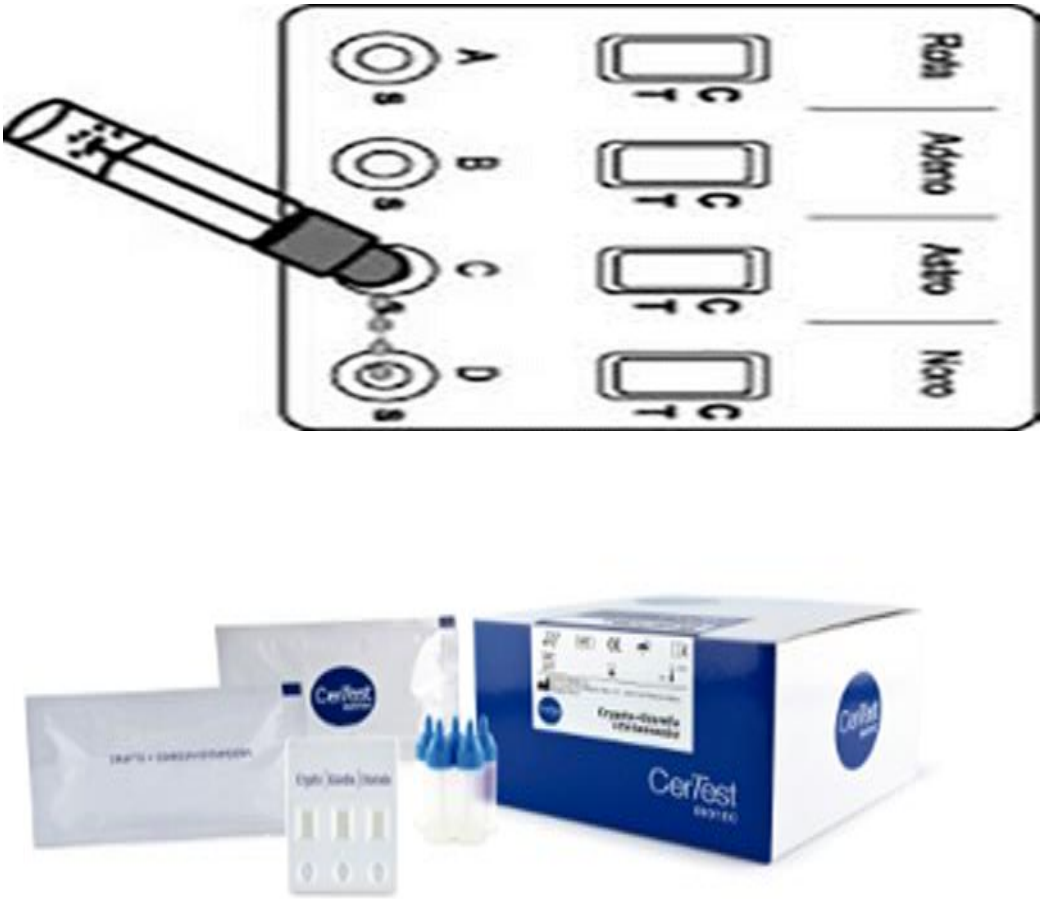

Fig. 2. Immunochromatographic tests

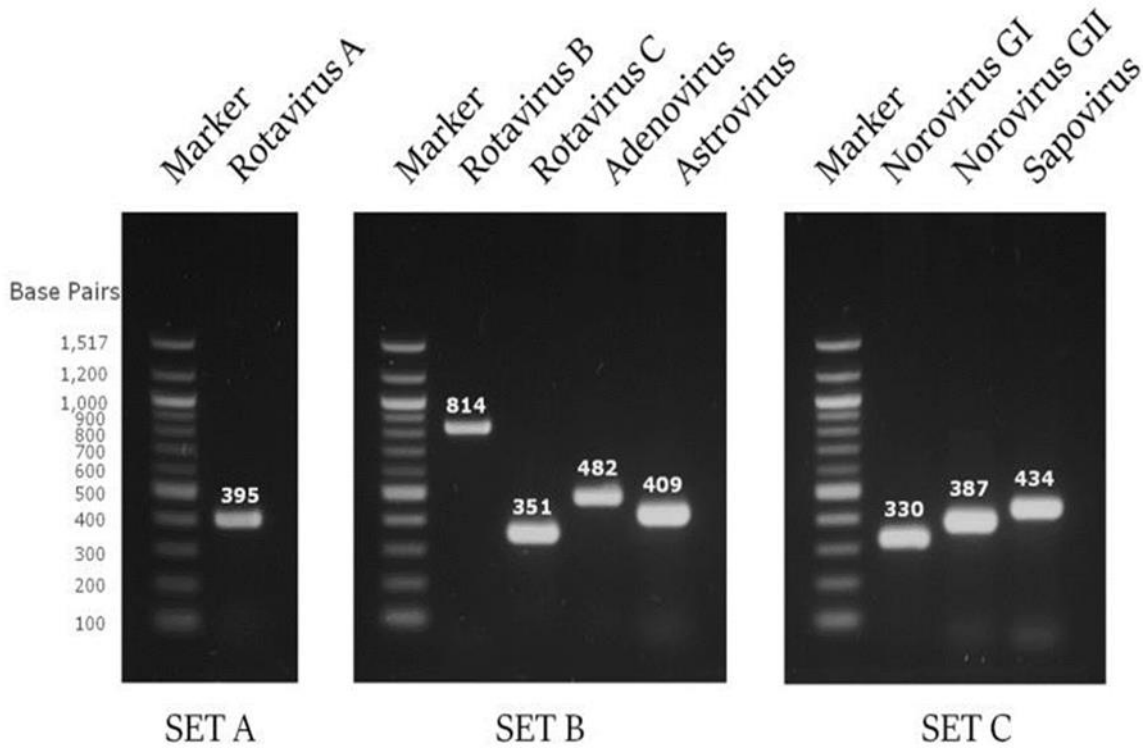

Fig.3. Real -Time PCR

2.3. Statistical methods:

Data were entered and processed with the statistical package IBM SPSS Statistics 23.0. A significance level at which the null hypothesis is rejected was $p<0.05$.

The following methods were applied:

1. Descriptive analysis - the frequency distribution of the considered signs, distributed by research groups, is presented in tabular form.

2. Analysis of Variance – to assess the characteristics of central tendency and statistical dispersion.

3. Graphical analysis – for visualization of the obtained results.

4. Alternative analysis – to compare relative shares.

5. Fisher's exact test and $\chi 2$ test - to test hypotheses about the presence of a relationship between categorical variables.

6. Anova - to test hypotheses for the presence of a relationship between quantitative variables.

III. RESULTS

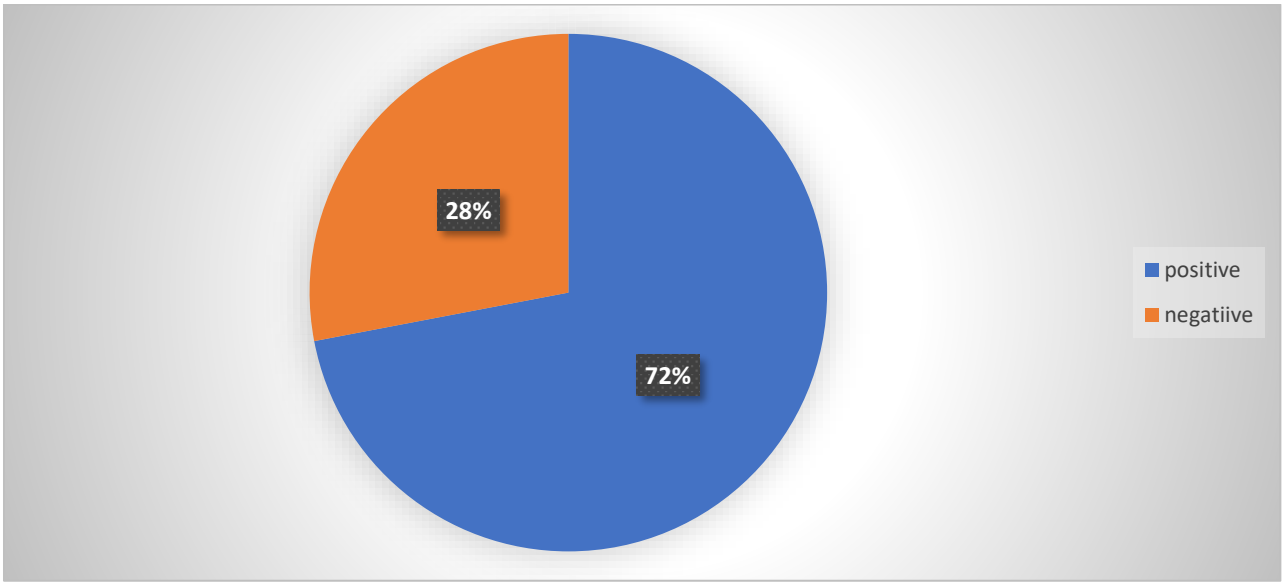

Fig. 4. Distribution of positive and negative IHT samples for viral intestinal infections

From fig.4. it is clear that of the 200 samples examined, 72% were positive and 28% negative for the intestinal viruses examined.

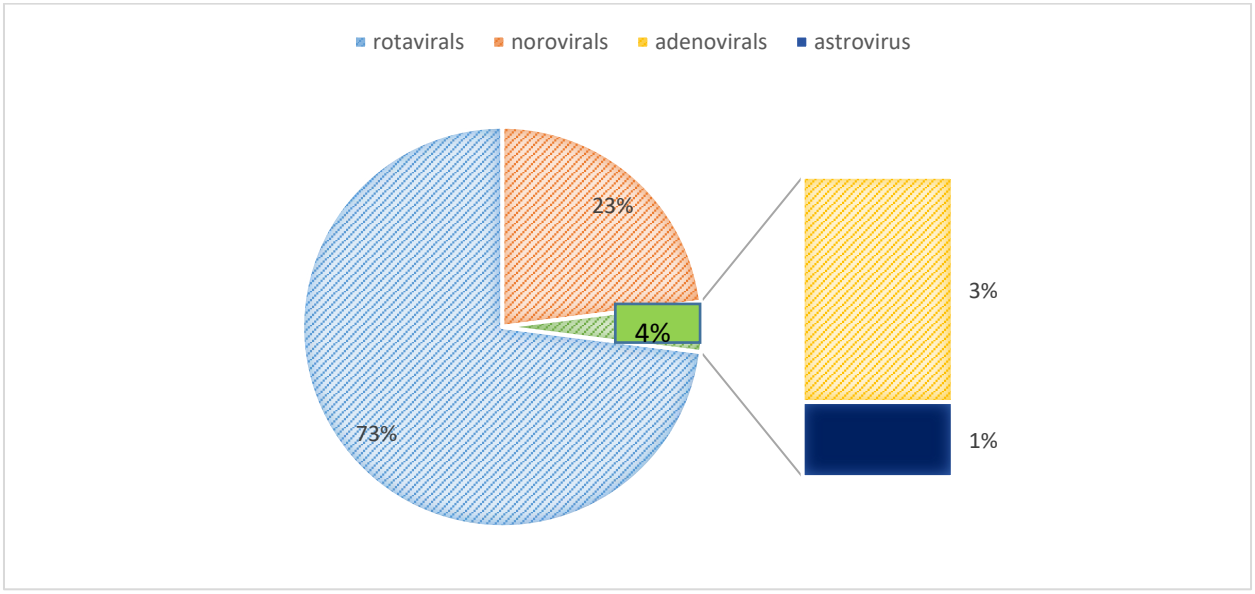

Fig. 5. Distribution of positive IHT for viruses according to the etiological agent

Fig. 5 shows that the relative share of rotavirus enteritis is the largest (73%), followed by noroviruses, adenoviruses are found in significantly fewer patients and astroviruses in a very small number, with unspecified viral intestinal infections are the remaining patients - 55 in number.

Table 1.Distribution of patients by gender according to etiological agents in number

Etiology agent	Man patient	Women patient
rotaviral 0-1 years	14	7
rotaviral 1-5 years	52	27
rotaviral 5-10 years	1	1
Rotaviral with vaccine 0-1years	0	0
Rotaviral with vaccine 1-5years	0	4
Rotaviral with vaccine 5-10 years	0	0
Noroviral l 0-1years	5	10
Noroviral 1-5years	9	2
Noroviral 5-10 years	2	5
Adenoviral 0-1 years	2	0
Adenoviral 1-5 years	1	0
Adenoviral 5-10 years	0	1
Astroviral 0-1 years	1	0
Astroviral-1-5years	1	0
Astroviral 5-10years	0	0

Viral intestinal unspecified 0-1years	4	2
Viral intestinal unspecified 1-5 years	20	8
Viral intestinal unspecified 5-10 years	8	13

Table 1 shows that there is a significant predominance of males in patients with rotavirus and norovirus enteritis in the age group of 1-5 years, a slight preponderance of females in the age group of 5-10 years in patients with norovirus enteritis. Generally finding male predominance. We observe a predominance of the male gender in the age group of 1-5 years in patients with unspecified viral intestinal infections.

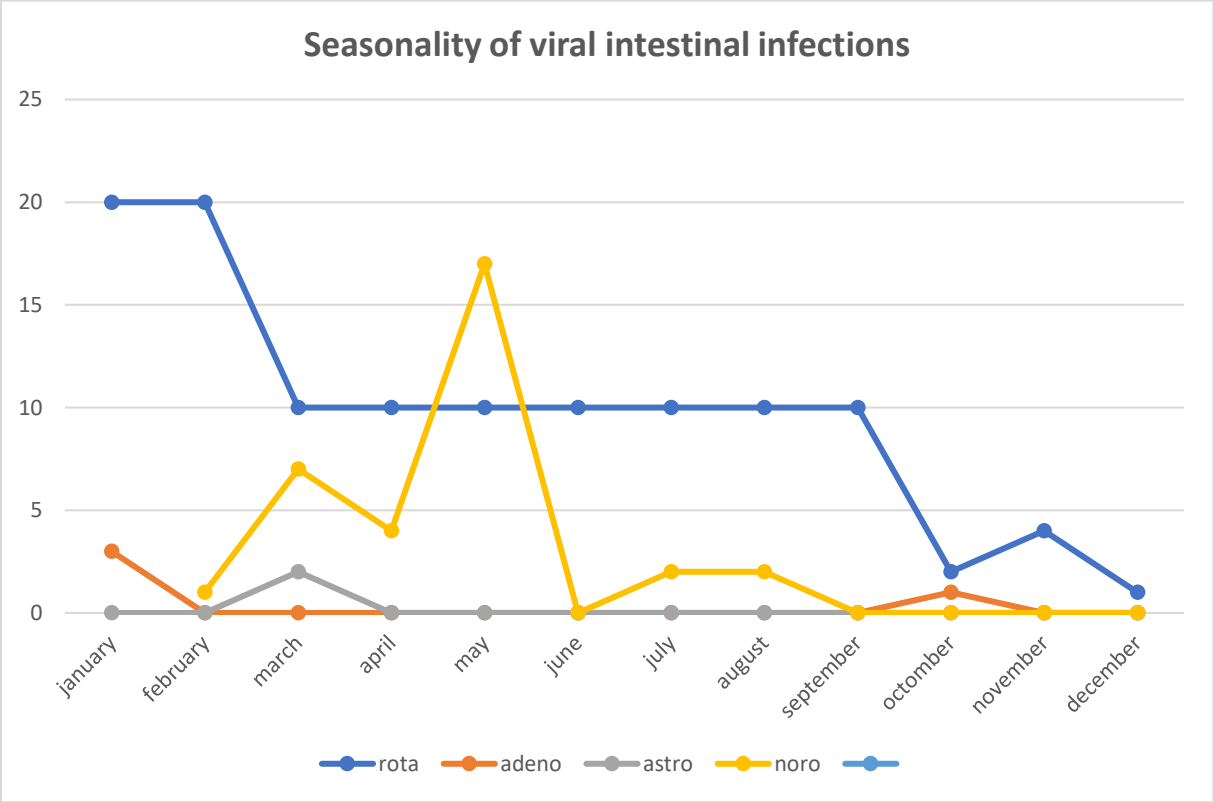

Fig. 6. Incidence of viral intestinal specified infections depending on seasonality (2018-2022)

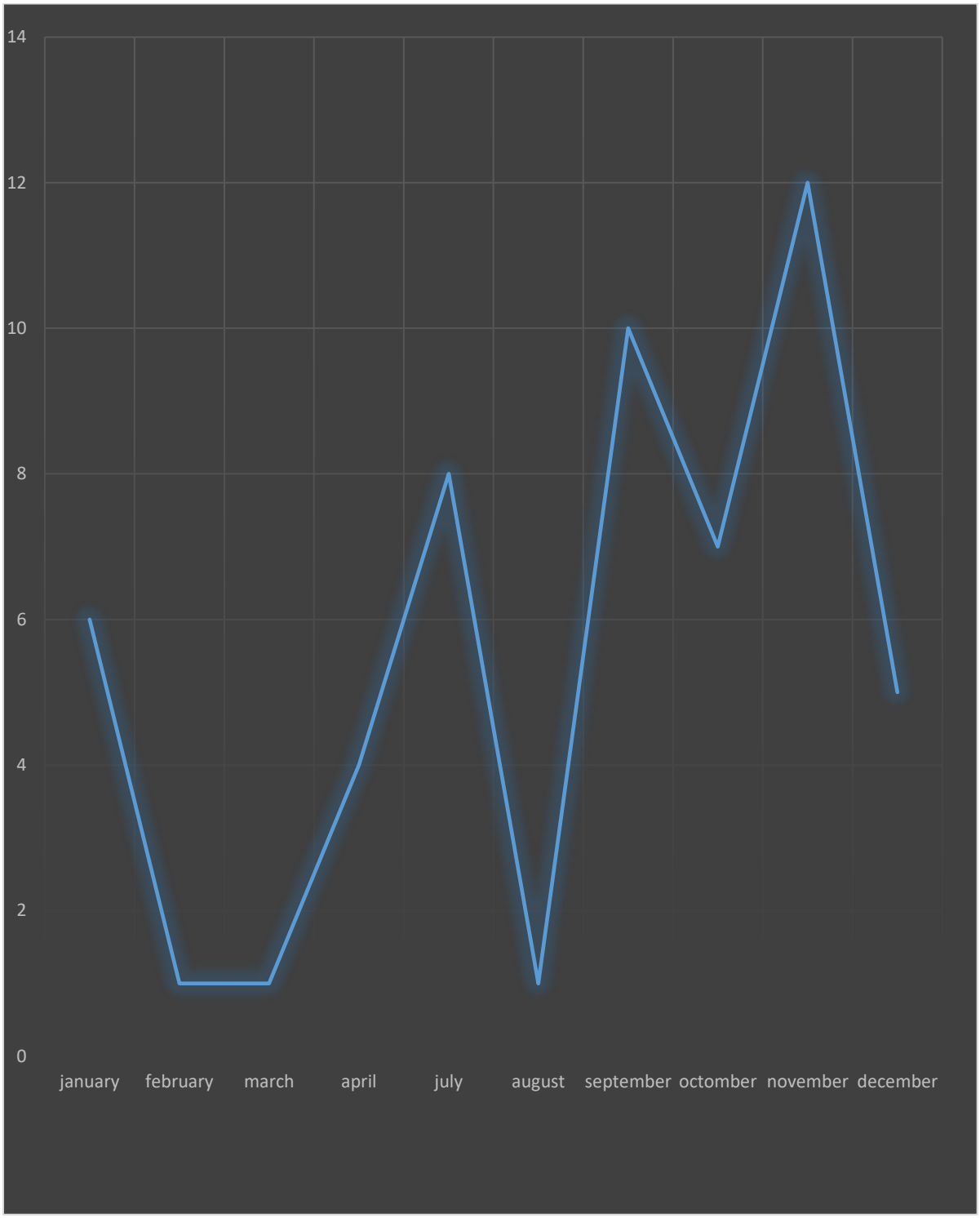

Fig. 7. Frequency of unspecified viral intestinal infections depending on seasonality (2018-2022)

It is clear from Fig. 6 that only norovirus infections have a distinct seasonality during the winter-spring period with a peak of the disease in the months of April-May.

From fig. 7 it is evident that the peaks of viral intestinal unspecified infections are mainly observed during the summer months of the year and the autumn season in the October-November period.

Demographic and clinical laboratory characteristics of unvaccinated rotavirus positive patients

Table 2. Frequency distribution of patients with rotavirus (unvaccinated) enteritis by age, sex, settlement and ethnicity

Age, Sex, Settlement, Ethnicity	Rotavirus enteritis n= 102
0-1 years	21(20,6%)
1-5 years	79(77,5%)
5-10 years	2(2%)
Male	6765,7%)
Female	35(34,3%)
City	92(90,2%)
Village	9(8,8%)
Bulgarian origin	86(84,3%)
Minority origin	16 (15,7%)

Table 2 shows that about two-thirds of the patients with rotavirus enteritis who have not been vaccinated for rotavirus are in the age group of one to five years - 79 of them are children.We observe a significant predominance of the male gender in children with rotavirus enteritis, not vaccinated for rotavirus, more than half -67 of them are boys. Majority of patients with rotavirus enteritis in urban residents - 90.2%, and the rural population is less than 10. Majority of sick children with rotavirus enteritis are of Bulgarian origin - 84.3%, and children from minorities are much less - 15.1%.

Table 3.Frequency distribution according to the symptoms of patients with rotavirus enteritis (non-vaccinated)

Symptoms		**n =102**	**in %-**
Nausea		14	13,8%
Vomiting		88	86.3%
Abdominal pain		3	2,9%
Diarrhea		100	98%
Pathological impurities		4	3,9%
Seizure symptoms		4	3,9%
Respiratory symptoms		17	15,7%

The symptom "nausea" is present in 14 of the children, and absent in more than ¾ of them.The data for the non-rotavirus-vaccinated, rotavirus-positive patients show that the symptom "vomiting" was present in 88 patients (86.3%), that is, it occurs in a large part of the sample. Shows that diarrhea is present in 100 (98%) of the patients and is absent in a minor part of the studied. Patients with rotavirus enteritis, unvaccinated for rotavirus, had the presence of blood in the stools. Convulsive symptoms were observed in four of the children with rotavirus enteritis, accounting for only 3.9% of cases, which represents a notably low percentage.

Table4.Frequency distribution of patients with rotavirus enteritis, unvaccinated for rotavirus, proven positive by immunochromatographic tests and real -time PCR

IHT	**PCR**
Positive patient-102	**Positive patient-102**
Negative patient-0	**Negative patients-0**

Table 4 prove that all positive immunochromatographic test patients for rotavirus enteritis were also proven positive by means of the Real Time PCR methodology. We have no statistically significant difference ($p \leq 0.05$).

Laboratory characteristics of rotavirus patients not vaccinated for rotavirus

Table 5. Minimum, maximum and average values of clinical and laboratory indicators in patients with rotavirus infections who were not vaccinated against rotavirus

	Leucocytes	**Hemoglobin**	**Platelets**	**ESR**
Mean	7,484	118,04	247,412	9,47
N	102	102	102	102
Std. Deviation	3,0560	10,383	93,6707	7,419
% of Total N	96,2%	96,2%	96,2%	96,2%
% of Total Sum	97,6%	96,1%	96,2%	97,4%
Minimum	2,5	92	28,9	5
Maximum	17,8	146	613,0	50
Median	6,950	117,00	235,500	7,00

Std. Error of Mean	,3026	1,028	9,2748	,735

From the above table, it can be seen that in patients not vaccinated against rotavirus, the average values of the main clinical and laboratory indicators do not show significant deviations from the norm.

Table6. Maximum, minimum, and average values of the ionogram in patients with rotavirus infections who were not vaccinated against rotavirus and were included in the study

		Potassium rates in rotavirus infections	Sodium rates in rotavirus infections	Chlorine rates in rotavirus infections
N	Valid	22	22	22
	Missing	0	0	0
Mean		4,2586	145,2682	105,3955
Std. Error of Mean		,07209	4,49176	,80750
Median		4,3000	141,4000	106,0000
Mode		4,30	142,00	99,00[a]
Std. Deviation		,33814	21,06822	3,78751
Range		1,55	106,00	11,00

Demographic and clinico-laboratory characteristics of rotavirus-vaccinated patients with rotavirus enteritis.

Table 7. Frequency distribution by age, sex, settlement and ethnicity of patients with rotavirus, vaccinated for rotavirus

Age, Sex, Settlement, Ethnicity	Rotaviral enteritis n= 4
0-1years	0(0%)
1-5years	4(100%)
5-10years	0(0%)
Male	0(0%)
Female	4(100%)
City	4(100%)
Village	0(0%)
Bulgarian origin	4(100%)
Minority origin	0(0%)

Table 7 indicates that patients who are vaccinated against rotavirus enteritis are only in the age group of one to five years. For previous years, there was no vaccine available. Rotavirus enteritis vaccination is recommended between 6 and 24 weeks, so it is perfectly normal for children under one year to also be vaccinated at a low rate or not at all, 100% of vaccinated children are female and there is not a single child that is vaccinated of male sex and 100% of vaccinated children are residents of cities and there is not a single one who was vaccinated and was from a village. All patients who were vaccinated for rotavirus enteritis were of Bulgarian origin. There is a lack of minority patients who are vaccinated against rotavirus.

Table 8. Frequency distribution according to the symptoms of patients with rotavirus enteritis, vaccinated for rotavirus

Symptoms:	**n =4**	**in %-100**
Nausea	2	50
Vomiting	2	50
Abdominal pain	2	50
Diarrhea	4	100

The symptom "nausea", as can be seen from the above table 8, occurs in half of the patients with rotavirus enteritis vaccinated for rotavirus, and in the remaining half it is not evident. The symptom "vomiting" was also present in half of the children with rotavirus enteritis vaccinated for rotavirus, and did not occur in the remaining 50%, abdominal pain was present in half of rotavirus-vaccinated children with rotavirus enteritis, that none of the children with rotavirus enteritis, vaccinated for rotavirus, had pathological impurities in the feces. Convulsive symptoms were not observed in patients with rotavirus enteritis vaccinated for rotavirus, respiratory symptoms are not detected in patients with rotavirus enteritis vaccinated for rotavirus.

Table.9 Frequency distribution of patients with rotavirus enteritis, vaccinated for rotavirus, proven positive by means of immunochromatographic tests and PCR

IHT	**PCR**
Negative patients-0	**Negative patients-0**
Positive patients-4	**Positive patients-4**

There is no statistically significant difference ($p \leq 0.05$). Therefore, from a cost-effectiveness point of view, rapid IHTs could also be used to prove rotavirus infections.

Laboratory characteristics of patients with rotavirus enteritis vaccinated for rotavirus

Table 10. Minimum, maximum and average values of clinical and laboratory indicators in patients with rotavirus infections who are vaccinated for rotavirus

	Leucocytes	Hemoglobin	Platelets	ESR
Mean	4,675	122,75	246,500	6,50
N	4	4	4	4
Std. Deviation	1,1206	8,382	11,2101	,577
% of Total N	3,8%	3,8%	3,8%	3,8%
% of Total Sum	2,4%	3,9%	3,8%	2,6%
Minimum	3,8	116	235,0	6
Maximum	6,3	135	260,0	7
Median	4,300	120,00	245,500	6,50
Std. Error of Mean	,5603	4,191	5,6051	,289

There are no significant deviations in the values of the clinical and laboratory indicators in the patients positive for rotavirus, but vaccinated for the disease.

We looked for whether there was a statistically significant difference between the number of defecations and length of hospital stay in patients with rotavirus enteritis, and whether there was a relationship between febrility values and length of hospital stay in patients with rotavirus enteritis, and also whether there was a difference in length of hospital stay in patients who are vaccinated against rotavirus and those who are not.

We did not find a statistically significant difference between the length of hospital stay and the number of bowel movements.

Hypotheses for a statistically significant difference are formed between equal parameters with only two alternatives. For example, between defecation in patients with and without vaccine. Rather, this type of hypothesis means that at each level of defecation the mean length of stay in days for all patients with that number is nearly the same or indistinguishable. Therefore, we preferred to look for functional dependence, how much defecation affects the stay in the hospital

From Fig.8 it is clearly seen that it is not possible to establish a clear functional relationship between the number of defecations and the length of hospital stay in relation to the number of defecations in patients with rotavirus gastroenteritis. For these unvaccinated patients, we have sufficient data.

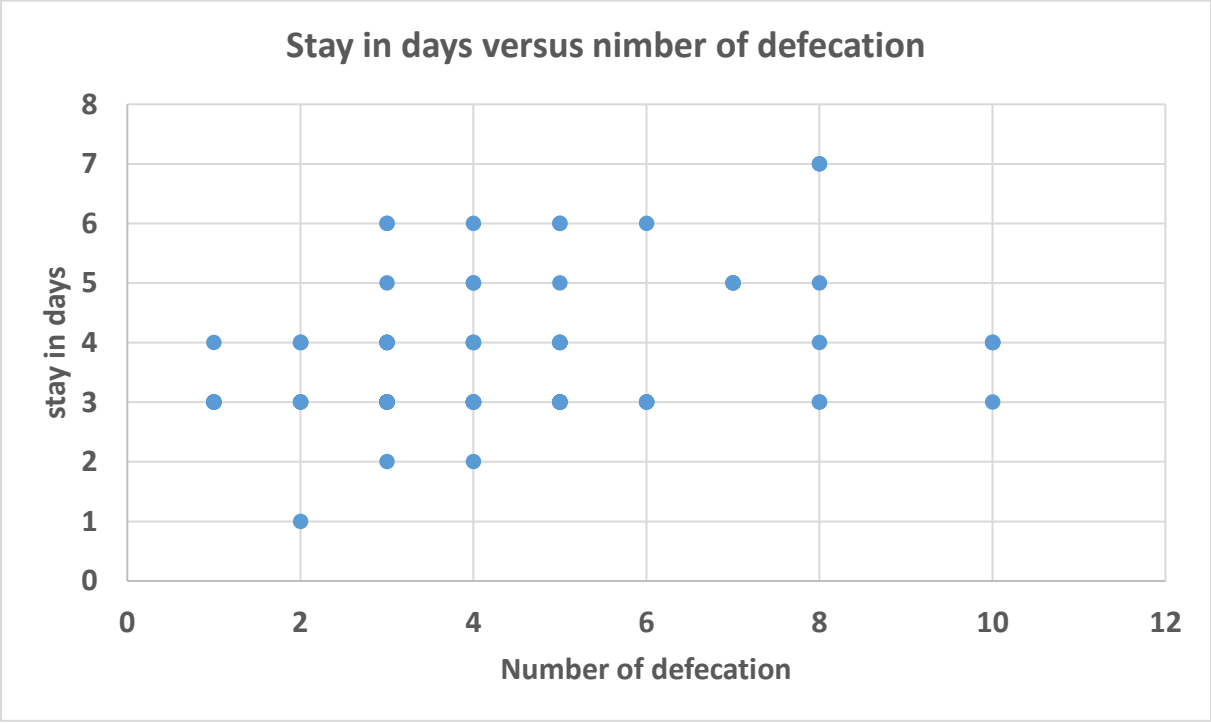

Fig. 8. Stay in days versus number of defecation in rotavirus gastroenteritis without vaccine

This can also be seen analytically, by trying to construct a linear regression. (Fig.9)

There is a certain tendency for the stay to increase with an increase in the number of defecations, but only 10% of the variation is explained by this (R^2=0.10. The parameters of the linear model (1) themselves are statistically significant with corresponding p_b=4.510^(-25) and p_a=0.000948 significantly below 0.05

y=ax+b=0.154947x+2.970516

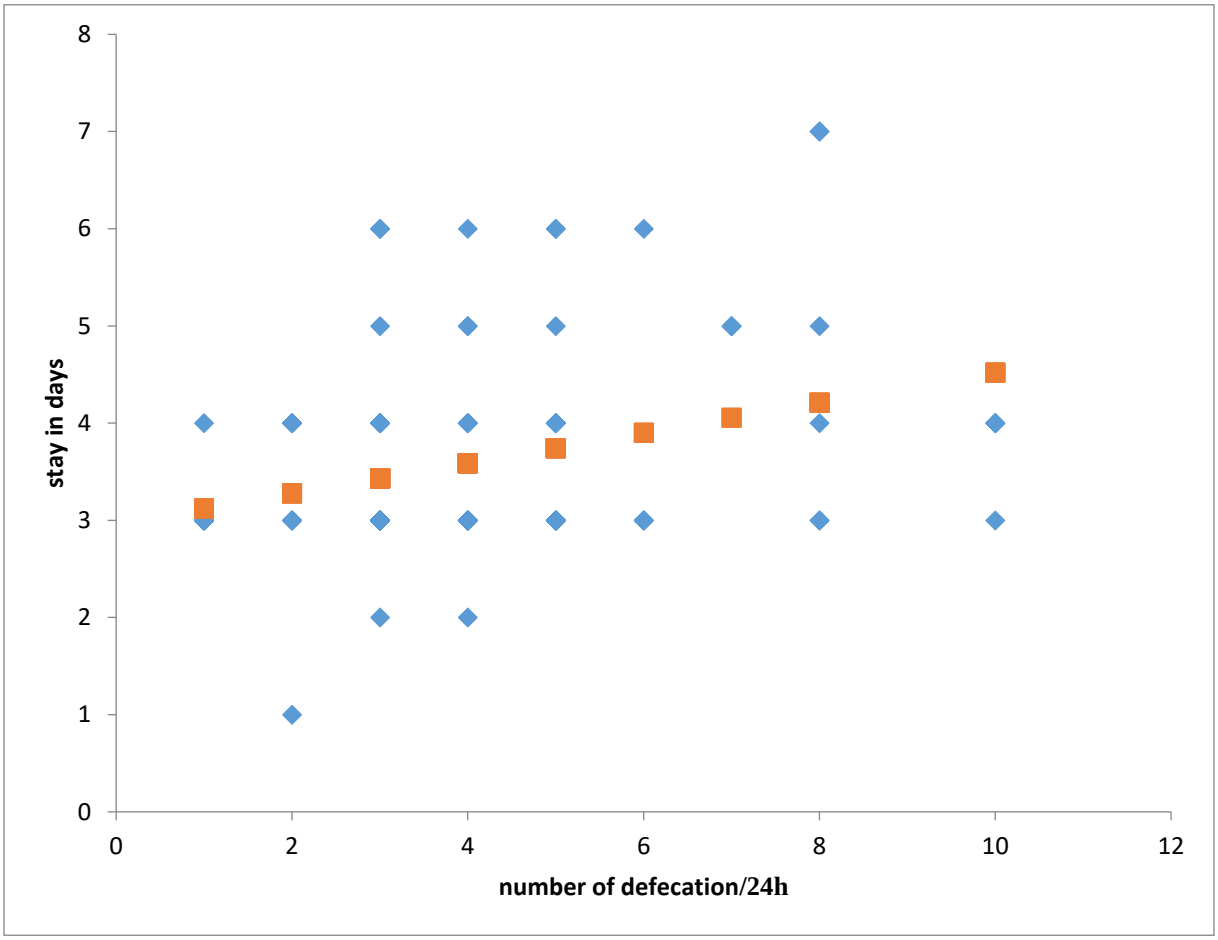

Fig. 9. Days of stay versus number of bowel movements in vaccine-free rotavirus gastroenteritis, linear regression

We also did not find a statistically significant difference between the length of hospital stay and fever values in patients with rotavirus infections (Fig. 10).

There is no such dependence, not even a trend. The regression does not explain any part of the variation (0.0008%) because there is not even a trend for increased length of stay relative to febrility

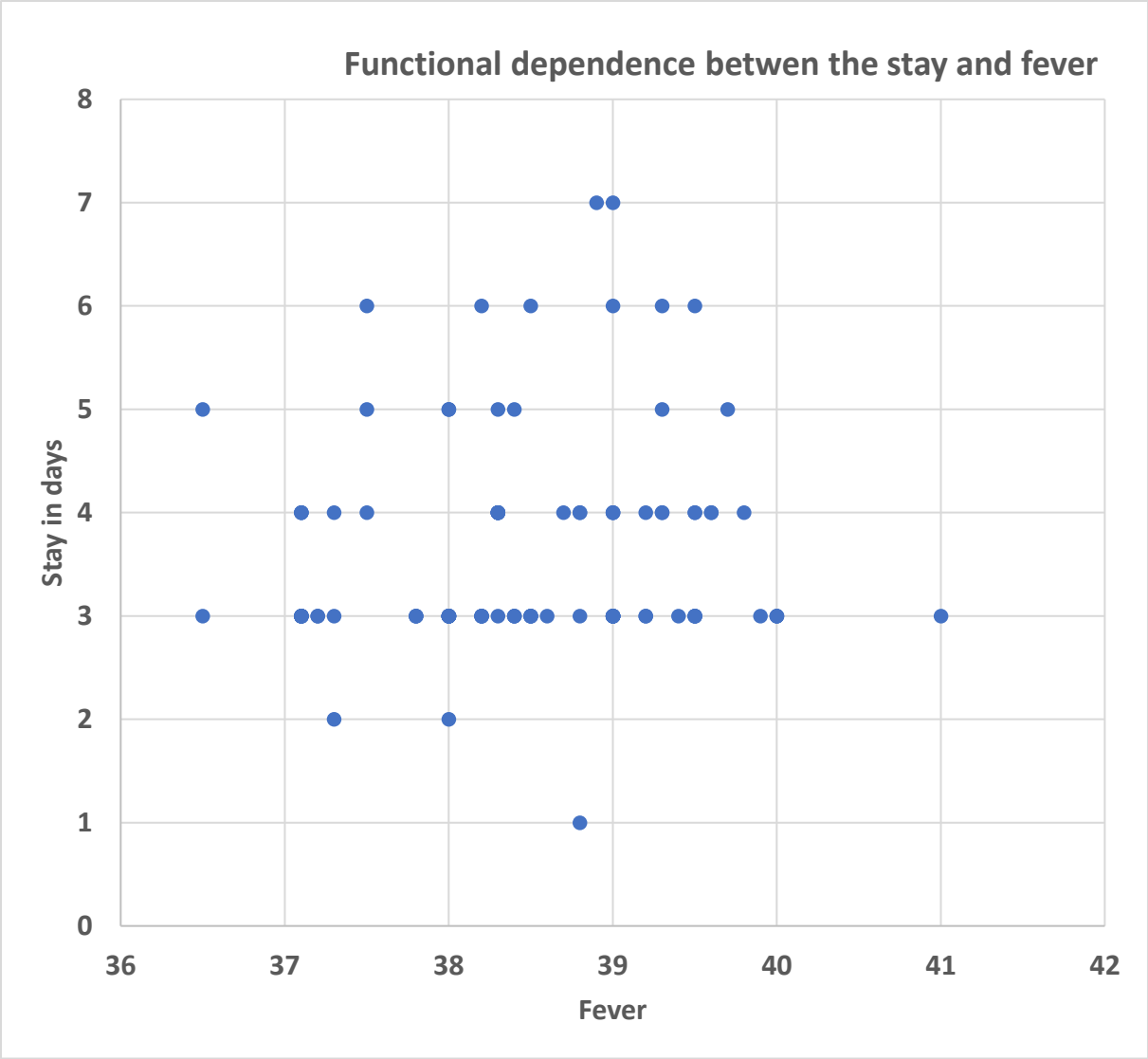

Fig. 10. There is no functional dependence between the stay and fever

We also found no statistically significant difference between the number of defecations in patients hospitalized for rotavirus enteritis with and without vaccine.

The average number of defecations without vaccine was 4.176470588 (102), with a standard deviation of 2.17279199, and with the vaccine: 2.75 (4), with a standard deviation of -0.5. There is no statistically significant difference here. This is a subpopulation where there is vaccine breakthrough. In the unvaccinated, the sick population is a much larger percentage of the exposed than in the vaccinated, so the sick vaccinated are not significantly different from the sick unvaccinated, but they are much less.

However, we found a statistically significant difference between the length of stay in days in patients vaccinated against rotavirus and the length of stay in days in patients not vaccinated against rotavirus.

Without vaccine, the average length of stay in days was 3.617647 (102), with a standard deviation of 1.044022, and for vaccine patients, 2.5 (4) with a standard deviation of 1.2910

The hypothesis that there is a significant difference between the two populations seems reasonable because $p < 0.0.409$.

Demographic and clinical laboratory characteristics of norovirus infections

Table 11.Frequency distribution by age, sex, settlement and ethnicity of patients with norovirus enteritis

Age, Sex, Settlement, Ethnicity	Noroviral enteritis n= 33
0-1 years	15(45%)
1-5 years	11(33%)
5-10 years	7(21%)
Male	15(45%)
Female	18(54%)
City	13(39,4%)
Village	20(60,6%)
Bulgarian origin	32(97%)
Ethnicity origin	1(3%)

From the table 11 it is evident that more than 1/3 of patients with norovirus enteritis are in the age group below one year, significant predominance of the female gender over the male gender in patients with norovirus enteritis, however, we note a slight predominance of the female gender, it is clear that the greater percentage of residents - 60% with norovirus enteritis - are residents of villages. We connect this mostly with the epidemic outbreak of norovirus enteritis in the territory of the village of Resilovo, municipality of Sapareva Banya, which occurred in the month of May 2022. From this outbreak were the majority of norovirus enteritis patients included in the study. Only one child of the patients with norovirus enteritis is of minority origin, and all the others are of Bulgarian origin - more than 97%.

Table 12. Frequency distribution according to the symptoms of patients with norovirus enteritis

Symptoms:	n =33	in %-100
Nausea	0	100%
Vomiting	16	48,5%
Diarrhea	16	48,5%
Respiratory symptoms	1	3%

That none of the children with norovirus enteritis reported nausea. Vomiting was observed as a symptom, however, in almost half of the examined patients with norovirus , that none of the children with rotavirus enteritis reported abdominal pain.None of the children with norovirus enteritis had pathological impurities. All have stools without the presence of blood and mucus. We have not registered convulsive symptoms in any of the children with norovirus enteritis.

Table 13. Frequency distribution of patients with norovirus enteritis, proven positive by means of immunochromatographic tests and PCR

IHT	**PCR**
Negative patients-0	**Negative patients-0**
Positive patients-33	**Positive patients-33**

Table 13 show that all patients who are positive for norovirus enteritis by IHT are also positive by Real Time PCR methodology.

Laboratory characteristics of patients with norovirus enteritis

Table 14. Maximum, minimum and average values of the main laboratory indicators of patients with norovirus enteritis.

		Leucocytes	**Platelets**	**ESR**
N	Valid	16	16	16
	Missing	0	0	0
Mean		5,4233	178,4545	4,1818
Median		,0000	159,0000	4,0000
Mode		,00	,00	,00
Std. Deviation		7,21382	199,76910	4,69344
Minimum		,00	,00	,00
Maximum		22,00	763,00	15,00

Note: The data are considered only for the 16 patients who were hospitalized, but the studies of non-hospitalized children with norovirus enteritis were not taken into account.

There were no significant deviations in the values of the main clinical and laboratory indicators in the patients with norovirus enteritis

Table 15. Maximum, minimum and average values of the ionogram of patients with norovirus enteritis

		Pottasium rate in noroviral infections	Sodium rate in noroviral infections	Chlorine rate in noroviral infection
N	Valid	5	5	5
	Missing	17	17	17
Mean		4,6860	140,6000	103,8800
Std. Error of Mean		,30476	1,63095	1,39800
Median		4,5000	139,0000	105,0000
Mode		4,03[a]	139,00	99,40[a]
Std. Deviation		,68145	3,64692	3,12602
Range		1,71	9,00	7,60
Minimum		4,03	138,00	99,40
Maximum		5,74	147,00	107,00

In Table 16, we have listed the values of the main indicators from the ionogram. We do not observe hypokalemia, unlike the patients with rotavirus infections.

We found a statistically significant relationship between the number of defecations in patients who were not hospitalized for norovirus enteritis and those who were ($p<0.05$), as can be seen depicted in Fig.11.

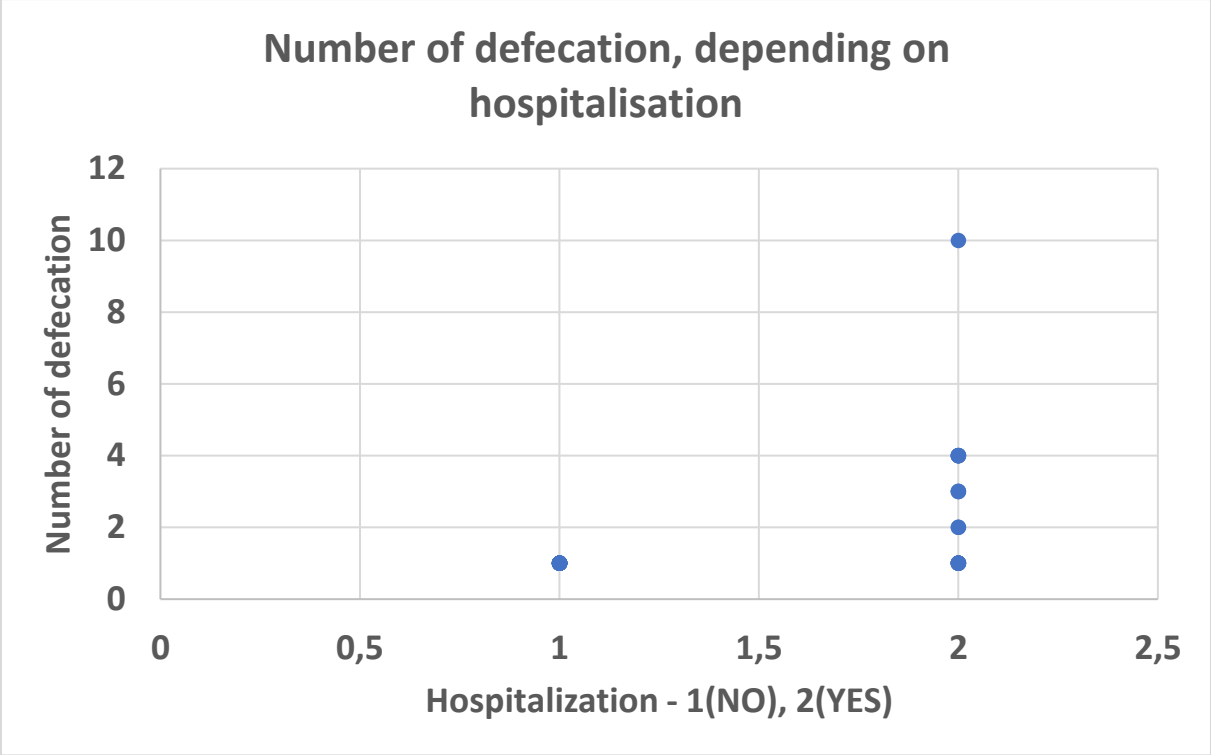

Fig. 11. Number of defecations, depending on hospitalization in patients with norovirus enteritis

A logistic regression can be done to show the relationship – Figure 12.

N-number of bowel movements

ln(P{not hospitalized}/P{hospitalized})=34.6955-33.9334.N (3)

P_value of both parameters is less than 10^(-51)

A logarithm of a number less than one is a negative number. As the number of bowel movements increases, the logarithm becomes negative and a smaller and smaller number, meaning that the probability of being hospitalized - P{hospitalized} is increasingly greater than that of not being - P{not hospitalize

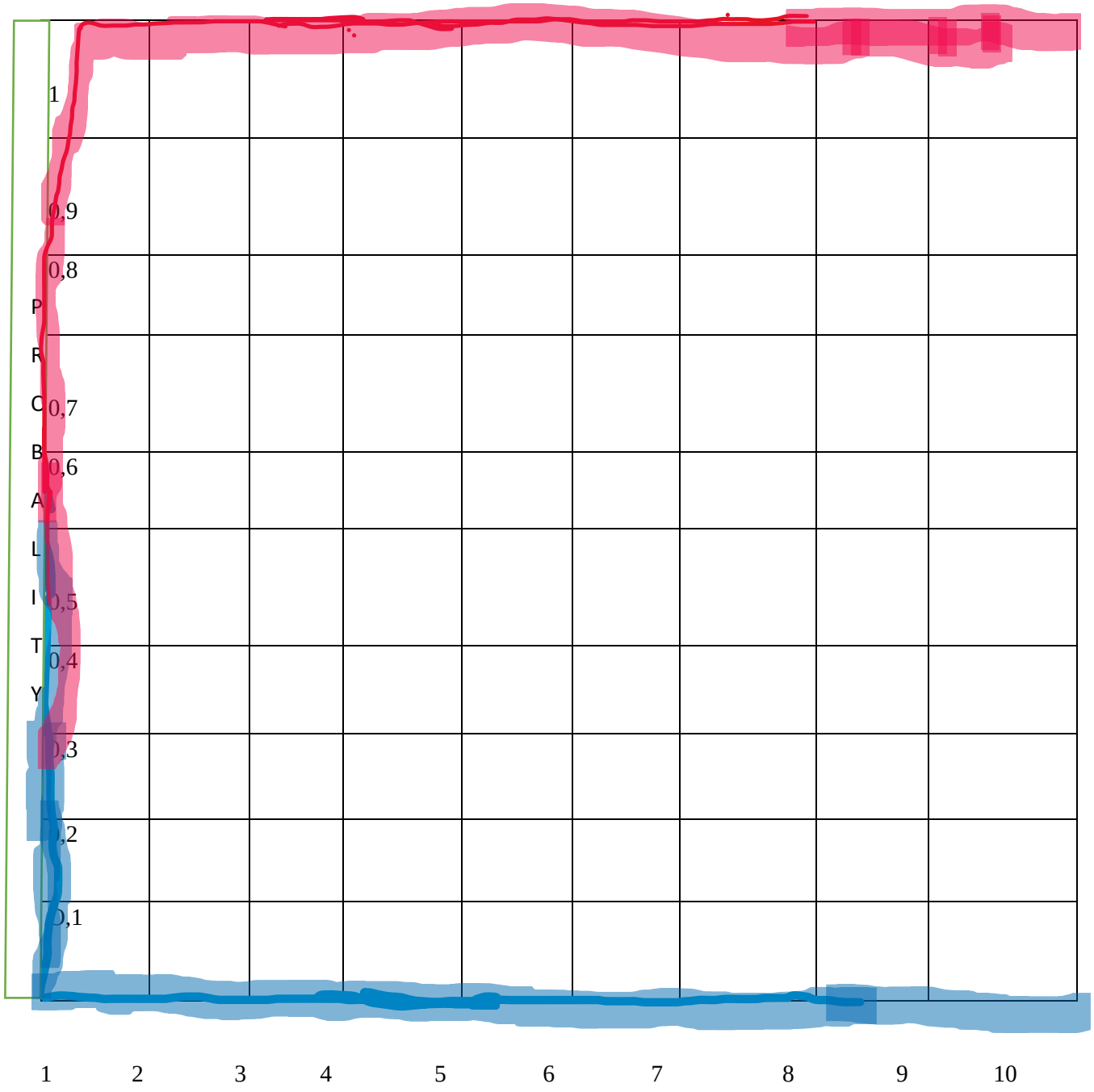

Fig.12.Relationship between the number of bowel movements and the probability of hospitalization

Demographic and clinical laboratory indicators of astrovirus infections

Table 16. Frequency distribution by age of patients with astrovirus enteritis

Age, Sex, Settlement, Ethnicity	Astroviral enteritis n= 2
0-1years	1(50%)
1-5years	1(50%)
5-10years	0(0%)
Male	2(100%)
Female	0(0%)
City	2(100%)
Village	0 (0)%)
Bulgarian origin	2(100%)
Military origin	0(0%)

It is clear from table 16 that the patients with astrovirus enteritis are in the age groups up to 5 years.

From table 16 it can be seen that 100% of the children included in the study with astrovirus enteritis were male. All patients with astrovirus enteritis were also urban residents, from table 16 it is also clear that all of the patients with astrovirus enteritis are of Bulgarian origin and there is not a single child of minority origin with astrovirus enteritis.

Table 17. Frequency distribution according to the symptoms of patients with astrovirus enteritis

Symptoms:	n =2	in %-100
Vomiting	1	50%
Diarrhea	2	100%

One of patitents with astrtovirus enteritis is with vomiting and both children are diarrhea, as shown tabl.17.

Table18.Frequency distribution of patients with astrovirus enteritis, proven positive by immunochromatographic tests and PCR

IHT	PCR
Negative patients -0	**Negative patients-1**
Positive patients -2	**Positive patients-1**

Table 18 show that only one of the children, proven positive with IHT, was also confirmed by the PCR method.

Laboratory characteristics of patients with astrovirus enteritis

Table 19. Maximum, minimum and average values of the main laboratory indicators of patients with astrovirus enteritis

		Leucocytes	Hemoglobin	Hct	Platelets	ESR
N	Valid	2	2	2	2	2
	Missing	2	2	2	2	2
Mean		6,4500	118,5000	,00	295,0000	10,5000
Std. Error of Mean		3,15000	6,50000	,000	40,00000	4,50000
Median		6,4500	118,5000	,00	295,0000	10,5000
Mode		3,30[a]	112,00[a]	0	255,00[a]	6,00[a]
Std. Deviation		4,45477	9,19239	,000	56,56854	6,36396
Minimum		3,30	112,00	0	255,00	6,00
Maximum		9,60	125,00	0	335,00	15,00

Table 19 makes it clear that there are no significant deviations in the values of the primary clinical and laboratory indicators among patients with astrovirus infections.

Demographic and clinical laboratory indicators of adenoviral infections

Tabl.20 Age, sex, settlement and ethnicity of patients with adenoviral enteritis

Age, sex, settlement and ethnicity	Adenoviral enteritis n= 4
0-1 years	2(50%)
1-5 years	1(25%)
5-10 years	1(25%)
Male	3(75%)
Female	1(25%)
City	4(100%)
Village	0(0%)
Bulgarian origin	4(100%)
Minority origin	0(0%)

From table 20 it is clear that the majority of children with adenovirus enteritis-3 are in the age group under five years and only one is in the age group over 5 years. We observe a significant prevalence of adenovirus enteritis in male children, only one of the children is female.All of the patients with adenovirus enteritis were from the cities, as shown in the table. 60. It is clear that 100% of the children with adenovirus enteritis are of Bulgarian origin and we have no children of minority origin in the examined patients with adenoviruses.

Tabl.21. Frequency distribution according to the symptoms of patients with adenoviral enteritis

Категории:	**n =4**	**в %-100**
Nausea	1	25%
Vomiting	4	100%
Abdominal pain	1	25%
Diarrhea	4	100%
Seizure	1	25%

Only one of the children reported nausea of all with adenoviral enteritis, and this was the child in the age group over 5 years. In the remaining 75% of children with adenoviruses, nausea was absent or could not be reported due to the fact that they were in the younger age groups.

As can be seen from table 21, vomiting was present in all patients with adenovirus enteritis. None of our examined children with adenovirus did not vomit. Abdominal pain was again observed in only one of the children with adenoviral enteritis, and was absent in the other 3. As shown in Table 21, diarrhea was also present in all patients with adenovirus enteritis. In none of the children with adenoviral enteritis, pathological impurities of blood and mucus were observed in the stools.

Convulsive symptoms were observed in only one of the 4 children with adenovirus gastroenteritis, which constitutes 25% of the entire examined group with adenoviruses. None of the children with adenoviral enteritis had respiratory symptoms during the study.

Table 22. Frequency distribution of patients with adenovirus enteritis, proven positive by means of immunochromatographic tests

IHT	**PCR**
Negative patients-0	**Negative patients -0**
Positive patients-4	**Positive patients-4**

Tables 22 show that all patients who were proven positive by IHT were also proven positive by Real Time PCR methodology.

Laboratory characteristics of patients with adenoviral enteritis

Table 23. Maximum, minimum and average values of the main laboratory indicators of patients with adenoviral enteritis

		Leucocytes	Hemoglobin	Platele	ESR
N	Valid	4	4	4	4
	Missing	0	0	0	0
Mean		10,2750	117,7500	293,2500	8,5000
Std. Error of Mean		1,44244	5,86480	72,58027	,95743
Median		9,9500	115,5000	277,5000	9,0000
Mode		7,10[a]	106,00[a]	140,00[a]	10,00
Std. Deviation		2,88487	11,72959	145,16054	1,91485
Minimum		7,10	106,00	140,00	6,00
Maximum		14,10	134,00	478,00	10,00

No significant deviations were observed in the values of the main clinical and laboratory indicators in the patients with adenoviral enteritis.

Demographic and clinical laboratory characteristics of patients with unspecified viral intestinal infections

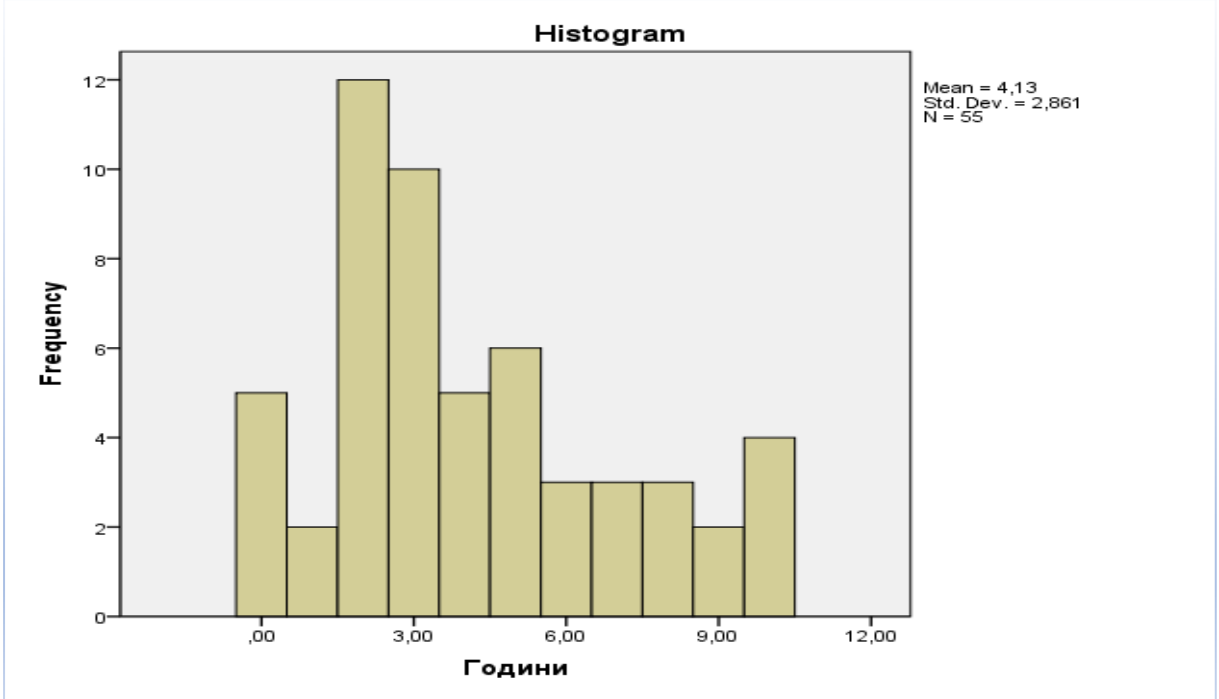

Fig. 13. Graphic representation of the distribution by years in the patients with unspecified viral intestinal infections

Table 24. Frequency distribution by age, sex, settlement and ethnicity of patients with unspecified viral intestinal infections

Age, sex, settlement and ethnicity	**Вирусни чревни неуточнени инфекции n= 55**
0-1 years	6(10,9%)
1-5 years	28(50,9%)
5-10 years	21(38,2%)
Male	32(58,2%)
Female	23(41,8%)
City	46(83,6%)
Village	9(16,4%)
Bulgarian origin	32(58,2%)
Minority origin	23(41,8%)

From table 24 it is clear that the main part of patients with viral intestinal unspecified infections are in the age group of 1-5 years, and the share of patients with viral intestinal unspecified infections in the age group 5-10 years is also significant. More than half of the children with unspecified intestinal

viral infections are male-32. The percentage of children with viral intestinal infections from the cities is significant - more than 2/3, and only 1/3 are from the villages. From table 24 it can be seen that the proportion of patients with unspecified viral intestinal infections of Bulgarian origin is greater, but with a negligibly low percentage of patients of minority origin, with the two groups approaching each other in terms of the incidence of unspecified viral intestinal infections.

Tabl.25.Frequency distribution according to the symptoms of patients with unsp. viral intestinal infections.

Categories:	**n =55**	**in %-100**
Nausea	1	1,8%
Vomiting	42	76,4%
Abdominal pain	28	50,9%
Diarrhea	53	94,5%
Respiratory symptoms	6	10.9%

Nausea was present in only one of the children with unspecified viral intestinal infections, all others did not report nausea, as is clear from the table.25. Vomiting prevails in over 2/3 of children with unspecified viral intestinal infections. Abdominal pains are present in more than half of our patients with unspecified viral intestinal infections. Diarrhea is present in over 90% of patients with unspecified viral intestinal infections and is absent in only three of the children. From table 25 it is clear that only six of the patients with unspecified viral intestinal infections have manifested respiratory symptoms, and over 80% of patients with unspecified viral intestinal infections have no such symptoms.

Table 26. Frequency distribution of patients with unspecified viral intestinal infections examined by means of immunochromatographic tests

IHT	**PCR**
Negative patients-55	**Negative patients-55**
Positive patients-0	**Positive patients-0**

Tables 26 show that all patients who were negative for viral intestinal infections by means of IHT were also negative by the PCR method.

Laboratory characteristics of patients with unsp. viral intestinal infections

Table 27. Maximum, minimum and average values of the main laboratory indicators of patients with unsp. viral intestinal infections

		Leucocytes	*Hemoglobin*	*Platelets*	*ESR*
N	*Valid*	*55*	*55*	*55*	*55*
	Missing	*2*	*2*	*2*	*2*
Mean		*9,8533*	*118,9455*	*312,6000*	*9,6364*
Std. Error of Mean		*,33658*	*1,92230*	*12,85374*	*,73643*
Median		*9,5900*	*122,0000*	*309,0000*	*8,0000*
Mode		*7,00[a]*	*129,00*	*189,00[a]*	*6,00*
Std. Deviation		*2,49613*	*14,25615*	*95,32587*	*5,46153*
Minimum		*2,50*	*80,00*	*104,00*	*3,00*
Maximum		*14,80*	*146,00*	*574,00*	*30,00*
Percentiles	*25*	*8,2000*	*108,0000*	*247,0000*	*6,0000*
	50	*9,5900*	*122,0000*	*309,0000*	*8,0000*
	75	*11,7000*	*129,0000*	*364,0000*	*11,0000*

No significant deviations were observed in the values of the main clinical and laboratory indicators in the patients with unspecified viral intestinal infections.

Table 28. Maximum, minimum, and average values of the ionogram in patients with noroviral enteritis

		Pottasium rate in unsp. viral intestinal infections	Sodium rate in unsp. viral intestinal infections	Chlorine rate in usp. viral intestinal infections
N	Valid	22	22	22
	Missing	0	0	0
Mean		4,5732	139,3545	104,2136
Std. Error of Mean		,28254	1,89569	1,01641
Median		4,4100	139,7000	104,5000
Mode		4,00[a]	139,00	103,00
Std. Deviation		1,32522	8,89155	4,76738
Range		6,85	45,00	22,30
Minimum		3,15	104,00	91,70
Maximum		10,00	149,00	114,00

Table 28 presents the values of trace elements in the patients with more severe dehydration that we have studied. Here, we have also recorded patients with hypokalemia, as well as those with hyperkalemia, but the average values of all trace elements in our patients with viral gastrointestinal non-specific infections do not differ from their normal values.

Patients with adenovirus infections, astrovirus infections and with rotavirus infections vaccinated against rotavirus are small in number, therefore it is not possible to statistically process them. Our patients with rotavirus infections (not vaccinated against rotavirus), norovirus infections and viral intestinal infections are sufficient in number to be able to perform statistical comparative analyzes between the different groups.

Comparative analysis of the groups with the different types of enteric viruses by categories of the examined clinical signs

Table.29.Comparative analysis of the course of rotavirus and norovirus gastroenteritis based on the categories of the investigated factors

Categories	Rotaviral enteritis		Noroviral enteritis		***P value***
	n =	%	n =	%	
Sex					
Male	92	90,2	15	45,5	**p<0,005**
Female	9	8,8	18	54,5	**p<0,005**
City	92	90,2	13	39,4	**p<0,005**
Village	9	8,8	20	60,6	**p<0,005**
Bulgarian origin	86	84,3	33	100	p>0,05
Minority origin	16	15,7	0	0	p>0,05
Abdominal pain	3	2,9	0	0	p>0,05
Nausea	14	13,7	0	0	p>0,05
Vomiting	88	86,3	31	93,9	p>0,05
Diarrhhea	100	98	16	48,5	**p<0,05**
Respiratory symptoms	8	7,8	1	3	p>0,05
Seizure	4	3,9	0	0	p>0,05

pathological impurities	4	3,9	0	0	p>0,05

Table 29 shows the established statistically significant dependencies concerning gender, location, and the symptom 'diarrhea' in patients with rotavirus and norovirus enteritis (Figures 14, 15, and 20). For all these categories of investigated factors, $p < 0.05$. A greater number of children with rotavirus enteritis are male, while the majority of patients with norovirus enteritis are female. The majority of children with rotavirus enteritis are residents of urban areas, while a higher percentage of children from rural areas have norovirus enteritis. The reason for this is the fact that our main group of patients with norovirus enteritis is part of an epidemic outbreak that occurred in a kindergarten in the village of Resilovo, Sapareva Banya municipality. We also observe a statistically significant difference regarding the symptom 'diarrhea,' with more than half of our norovirus enteritis patients lacking this symptom.

Table 30.Comparative analysis of the course of rotavirus enteritis and viral gastrointestinal unclassified infections based on the categories of the investigated factors."

Categories	Rotaviral enteritis		Viral intestinal unsp. infections		**P value**
	n =	%	n =	%	P value
Sex					
Male	92	90,2	30	54,5	**p<0,05**
Female	9	8,8	25	45,4	**p<0,05**
City	92	90,2	46	83,6	p>0,05
Villagr	9	8,8	9[a]	16,4	p>0,05
Bulgarian origin	86	84,3	32	58,2	p>0,05
Minority origin	16	15,7	23	41, 8	p>0,05
Abdominal pain	3	2,9	28	50,9	**p<0,05**
Nausea	14	13,7	1	1,8	**p<0,05**
Vomiting	88	86,3	42	76,4	p>0,05
Diarrhea	100	98	52	94, 5	p>0,05
Respiratory symptoms	8	7,8	6	10,9	p>0,05
Seizure	4	3,9	0	0	p>0,05

Pathological impurites	4	3,9	0	0	p>0,05

.

Table 30 illustrates the presence of statistically significant dependencies concerning gender and the symptoms 'abdominal pain' and 'nausea' in patients with rotavirus infections and viral gastrointestinal unclassified infections (Figures 14, 17, and 18). For all these categories, $p < 0.05$. A greater proportion of male patients is significant in cases of rotavirus enteritis, while a larger proportion of female patients is significant in cases of viral gastrointestinal unclassified infections. A significantly higher percentage of children with viral gastrointestinal unclassified infections have abdominal pain, while a significantly higher percentage of children with rotavirus enteritis experience nausea.

Table31.Comparative analysis of the course of norovirus enteritis and viral gastrointestinal unclassified infections

Categories	Noroviral enteritis		Viral intestinal infections		***P value***
	n =	%	n =	%	
Sex					
Male	15	45,5	30	54,5	p>0,05
Female	18	54,5	25	45,4	p>0,05
City	13	39,4	46	83,6	**p<0,05**
Village	20	60,6	9[a]	16,4	**p<0,05**
Ethnicity	33	100	32	58,2	**p<0,05**
Bulgarian origin	0	0	23	41, 8	**p<0,05**
Abdominal pain	0	0	28	50,9	**p<0,05**
Nausea	0	0	1	1,8	p>0,05
Vomiting	31	93,9	42	76,4	p>0,05
Diarhhea	16	48,5	52	94, 5	**p<0,05**
Respiratory symptoms	1	3	6	10,9	p>0,05
Seizure	0	0	0	0	p>0,05
Pathological impurities	0	0	0	0	p>0,05

Table 31 shows that we have identified a statistically significant relationship concerning the place of residence, ethnicity, symptoms of abdominal pain, and diarrhea in patients with norovirus enteritis and viral gastrointestinal unclassified infections (Figures 15, 16, 17, and 20). A larger proportion of children with viral gastrointestinal unclassified infections are residents of urban areas, while a significantly larger proportion of patients from rural areas have norovirus enteritis, which can be attributed, as mentioned in the earlier comparative analysis between rotavirus and norovirus enteritis, to the fact that the majority of our patients with norovirus enteritis are part of the epidemic outbreak that occurred in the kindergarten in the village of Resilovo.

Only one of the children with norovirus enteritis is of a minority background, while the rest are of Bulgarian ethnicity. Apparently, a more significant proportion of patients from minority backgrounds have viral gastrointestinal unclassified infections.

The symptoms of abdominal pain and diarrhea are more commonly encountered in patients with viral gastrointestinal unclassified infections, and more children with norovirus enteritis lack the symptom of diarrhea and have not reported abdominal pain. For all the above-mentioned categories in patients with norovirus enteritis and viral gastrointestinal unclassified infections, $p < 0.05$.

The data provided in the tables below are also represented graphically:

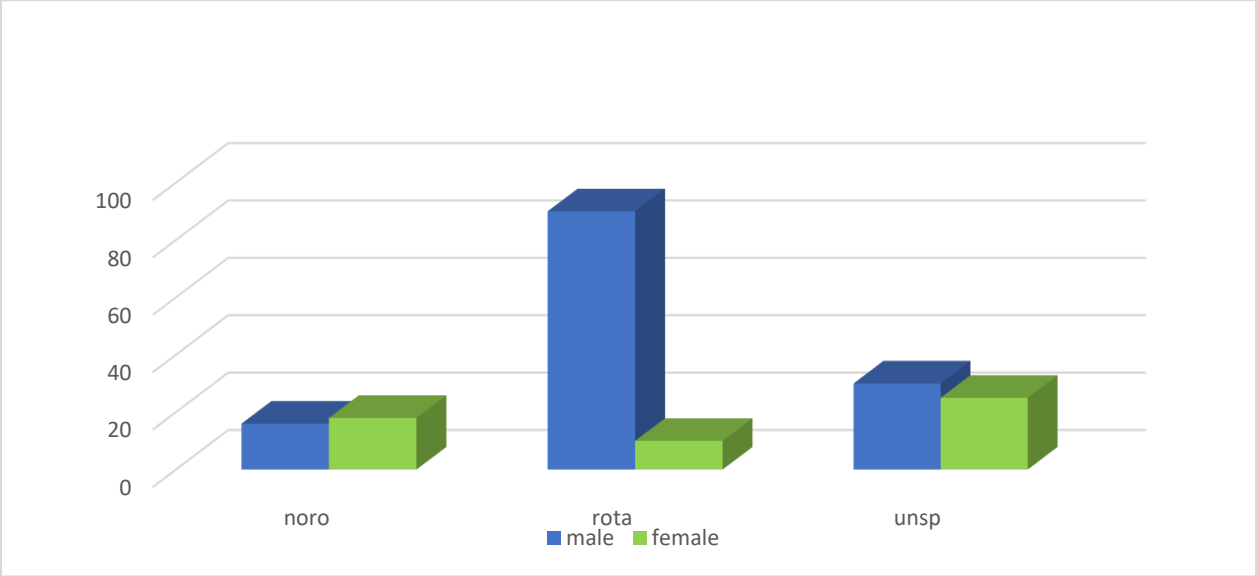

Fig. 14. Comparative analysis of the studied groups by gender

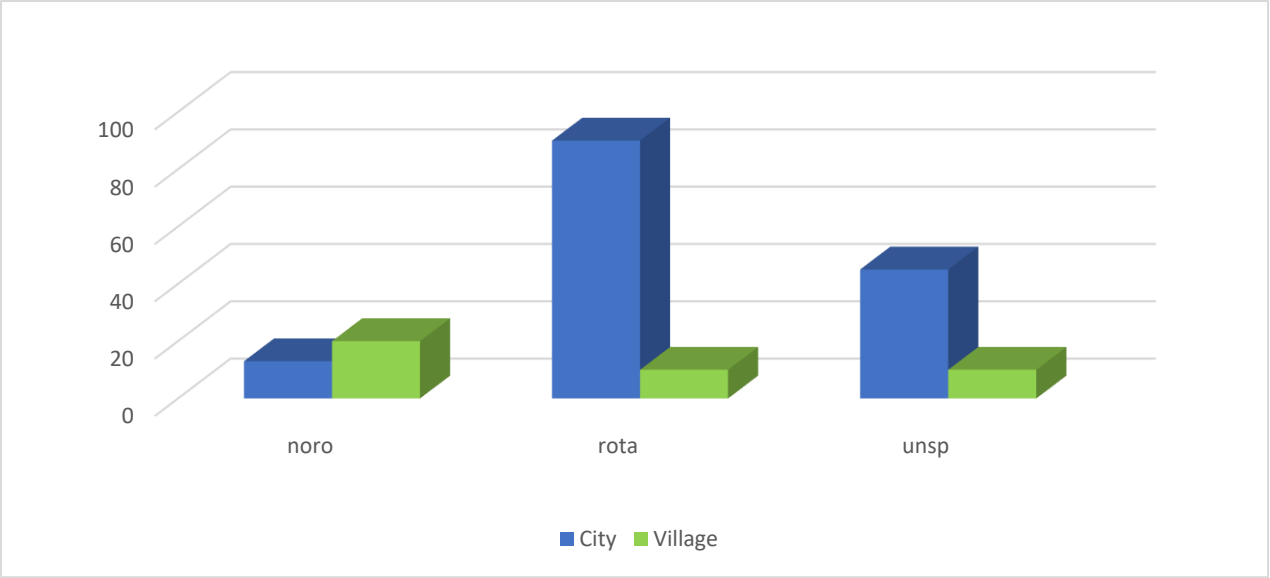

Fig. 15. Comparative analysis of the studied groups by settlement

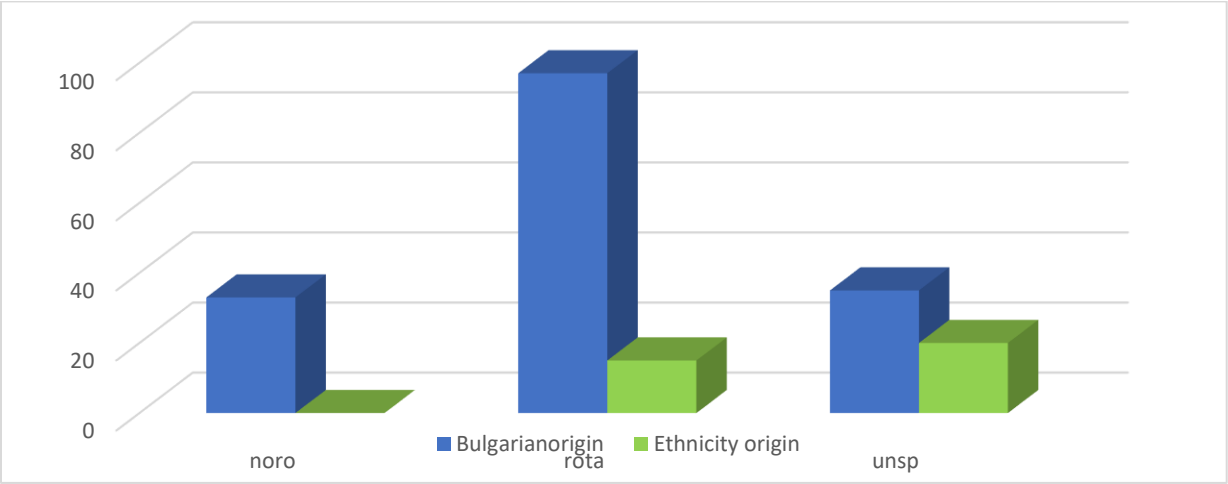

Fig. 16. Comparative analysis of the studied groups by ethnicity

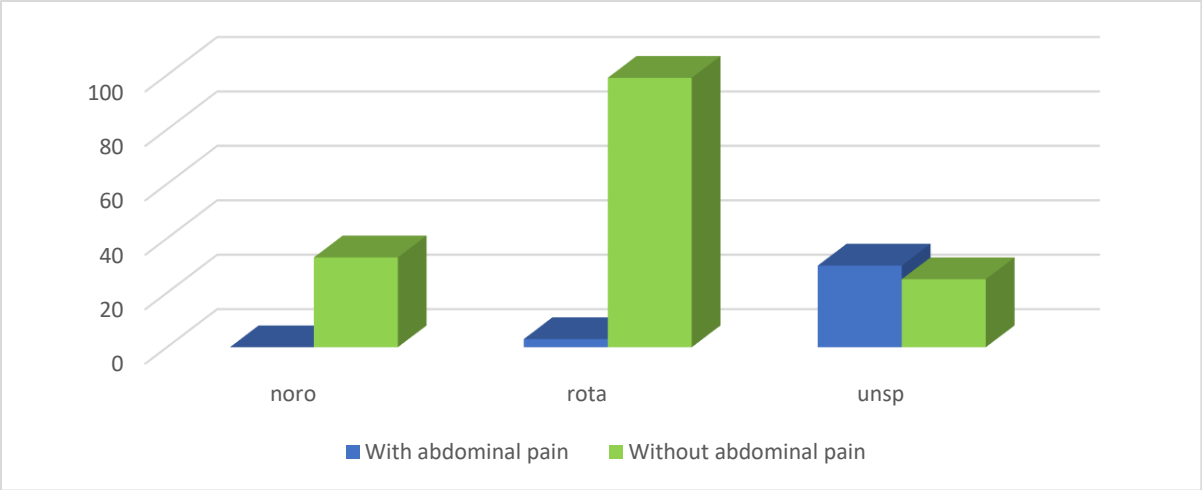

Fig. 17. Comparative analysis of the studied groups according to the symptom "abdominal pain”

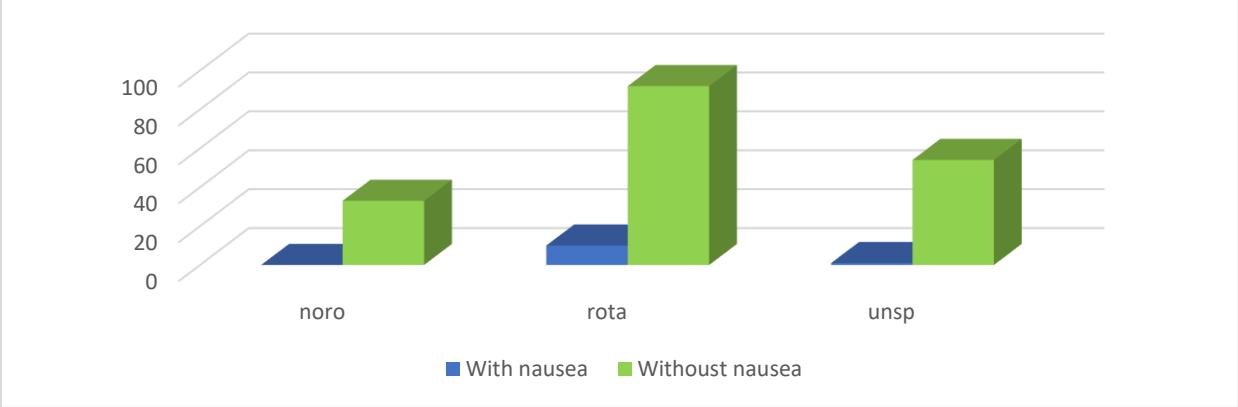

Fig. 18. Comparative analysis of the studied groups according to the symptom "nausea"

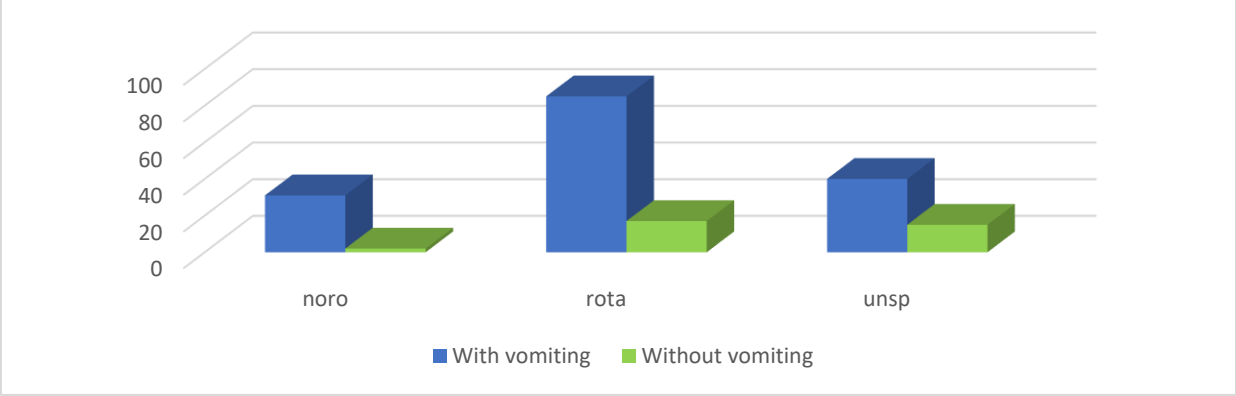

Fig. 19. Comparative analysis of the studied groups according to the symptom "vomiting"

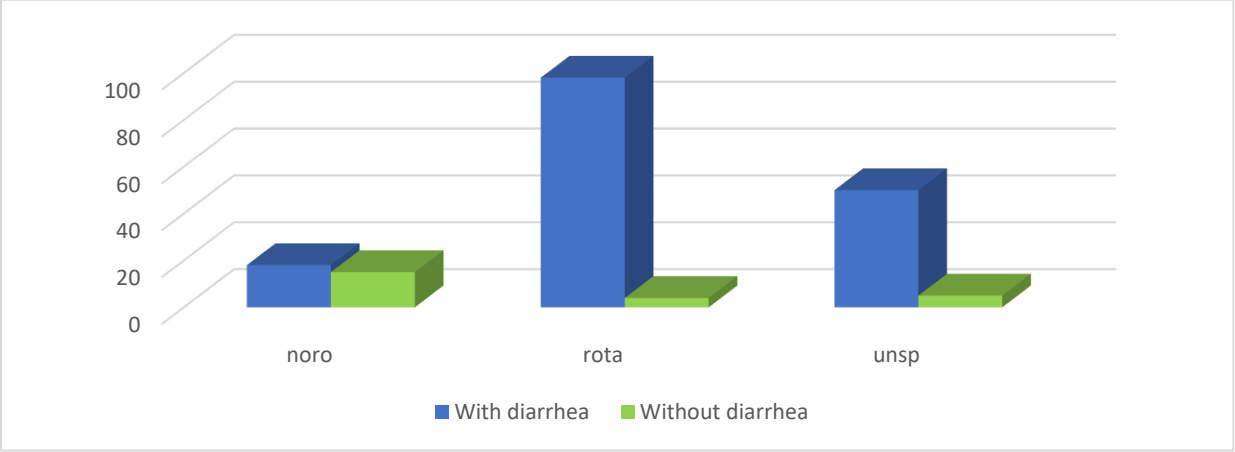

Fig. 20. Comparative analysis of the studied groups according to the "diarrhea" symptom

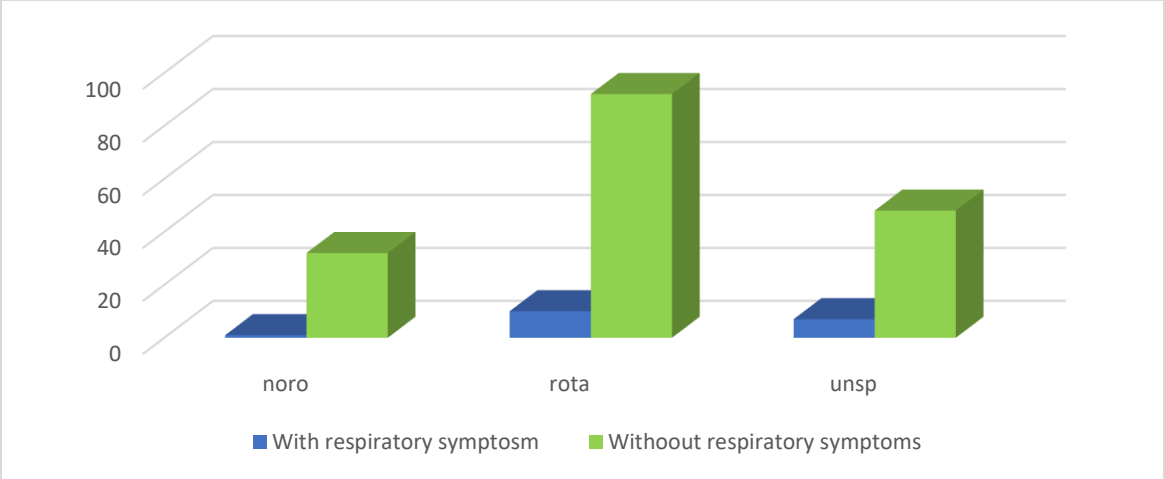

Fig. 21. Comparative analysis of the studied groups by respiratory symptoms

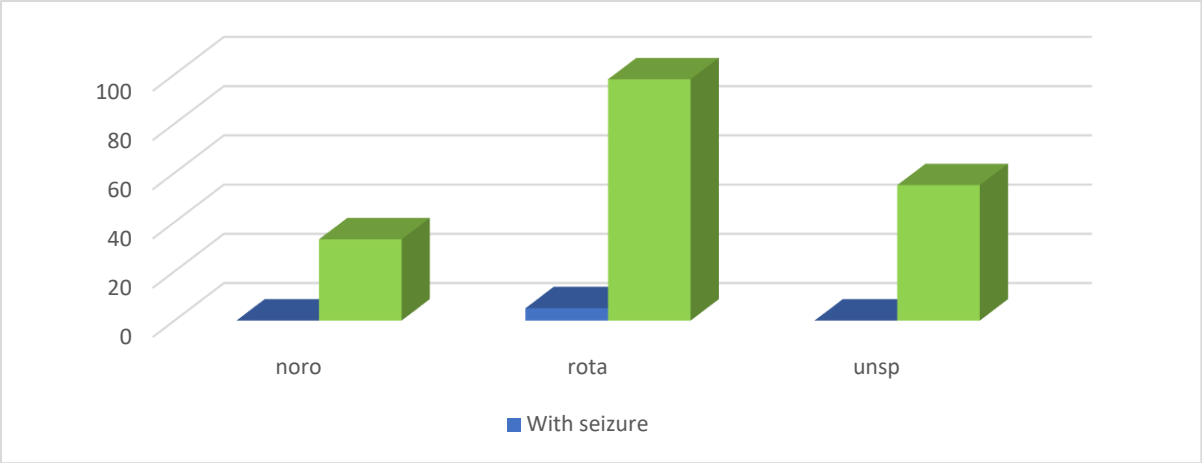

Fig. 22. Comparative analysis of the studied groups according to seizure symptoms

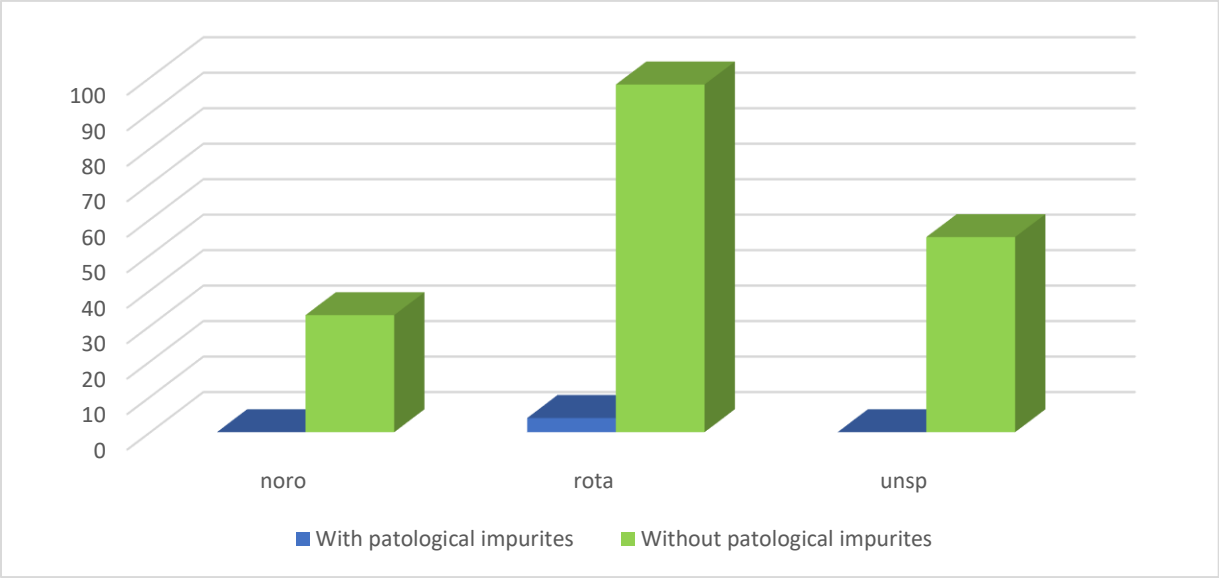

Fig. 23. Comparative analysis of the studied groups according to the presence of pathological impurities in the feces

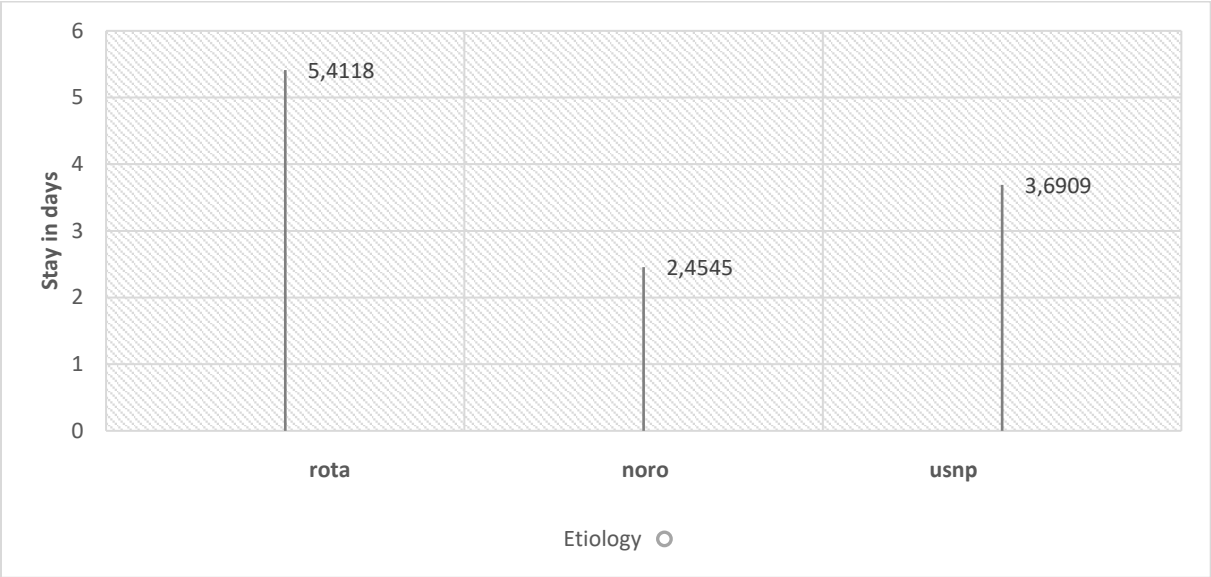

Fig. 24. Comparative analysis of length of hospital stay in patients with rotavirus enteritis, unvaccinated for rotavirus, patients with norovirus enteritis and those with unspecified viral intestinal infections

We found a statistically significant difference in length of hospital stay depending on the etiological agent.

The mean hospital stay for patients with rotavirus enteritis was 5.4118, with a standard deviation of 1.70237, for patients with norovirus enteritis 2.4545 with a standard deviation of 1.60255, and for patients with unspecified viral intestinal infections 3.6909 with a standard deviation of 1 ,03410($p<0.05$). The hospital stay was the longest in patients with rotavirus enteritis, and the shortest in patients with norovirus enteritis.

We also observed a statistically significant difference in the maximum number of defecations compared to the etiological agent ($p<0.05$). The data, including the average values of the maximum number of defecations, as well as their standard deviations, are shown in the table. 87. The average value of the maximum number of defecations is the largest in rotavirus enteritis, and the smallest in norovirus:

Table 32. Average values by etiological agents of the maximum number of defecation

Etiology agent		Maximum defecation for 24 hours
rota	**Mean**	4,1765
	N	102
	Std. Deviation	2,17279
noro	**Mean**	1,9697
	N	33
	Std. Deviation	1,84535
Unsp.	**Mean**	3,7273
	N	55
	Std. Deviation	2,00420
Total	**Mean**	3,6632
	N	190
	Std. Deviation	2,21161

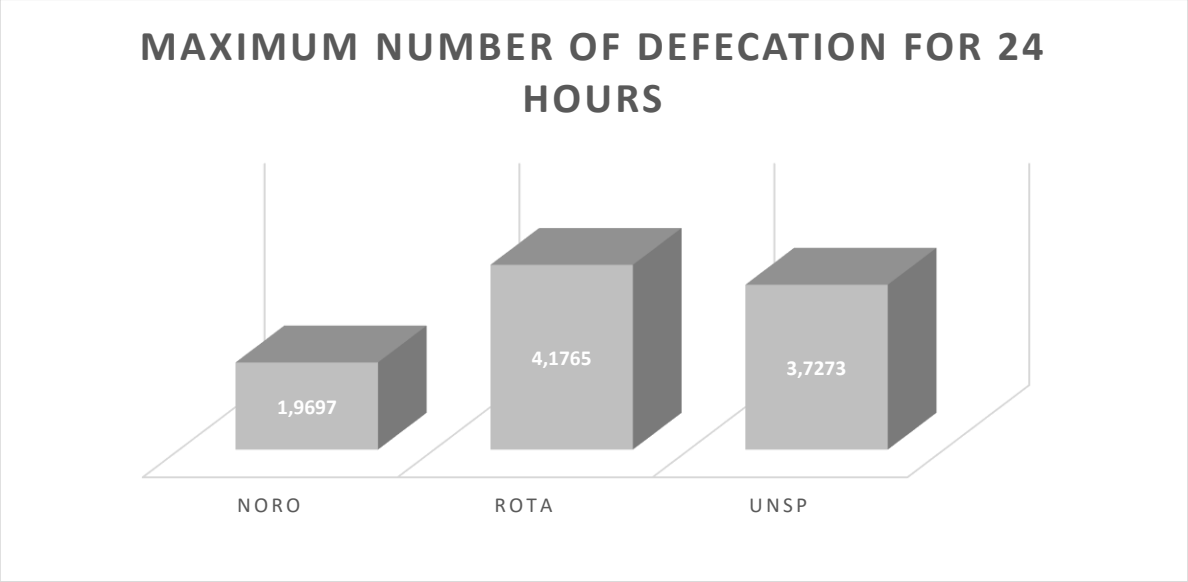

Fig. 25. Comparative analysis of the average value of the maximum number of defecations by etiological compared agents, graphical presentation

Table 33. Average values of febrility by etiological agents:

Etiological agent		Temperature 0C
Rotaviral	**Mean**	38,4549
	N	102
	Std. Deviation	,88883
Noroviral	**Mean**	36,7939
	N	33
	Std. Deviation	,86636
Unsp.	**Mean**	37,2600
	N	55
	Std. Deviation	1,02136
Total	**Mean**	37,8205
	N	190
	Std. Deviation	1,15756

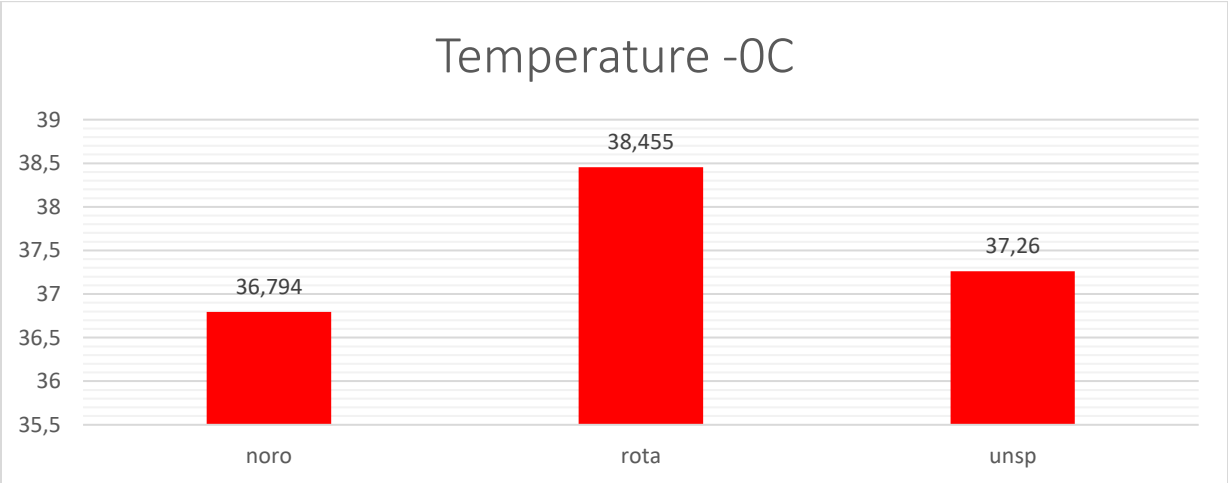

Fig.26. Comparative analysis in the mean values of febrility by etiological agents

We also observe a statistically significant difference in the mean values of febrility by etiological agents ($p<0.05$). We report the highest values of fever in patients with rotavirus enteritis, and the lowest in patients with norovirus enteritis, the average value of fever in patients with viral intestinal, unspecified infections approaches subfebrile ones.

We also observed a statistically significant difference in two of the main clinical and laboratory indicators - leukocytes and platelets compared to the etiological agents ($p<0.05$). We did not find a statistically significant difference in terms of hemoglobin and erythrocyte sedimentation rate (ESR) in relation to etiologic agents ($p>0.05$).

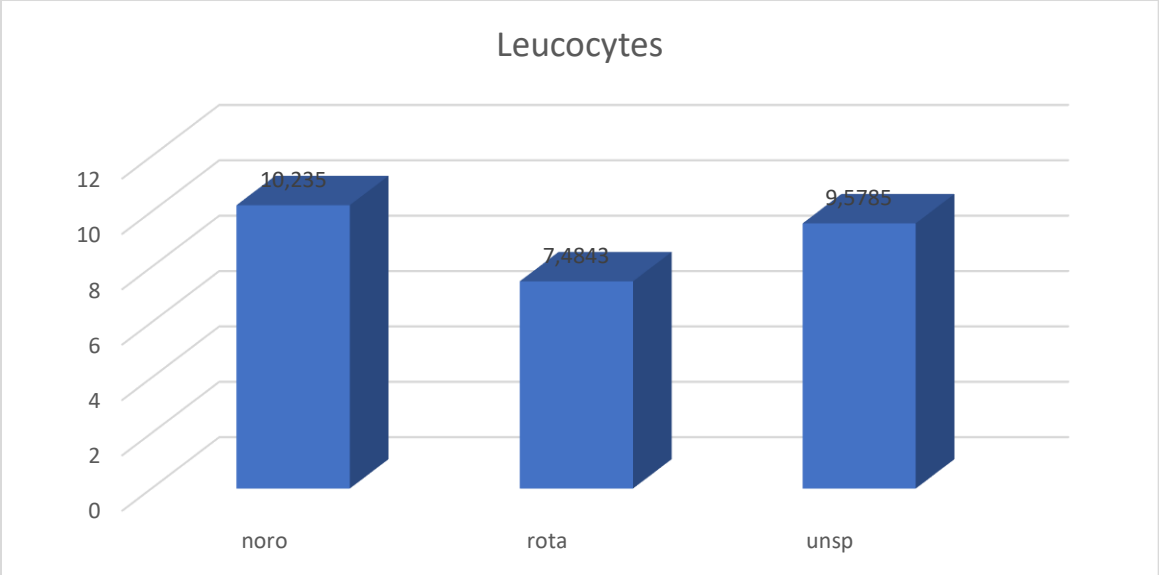

Fig. 27. Comparative analysis of the average values of leukocytes in relation to etiological agents

From fig. 27 it can be seen that the average value of leukocytes is the highest in patients with norovirus gastroenteritis and the lowest in patients with rotaviruses not vaccinated for rotavirus enteritis.

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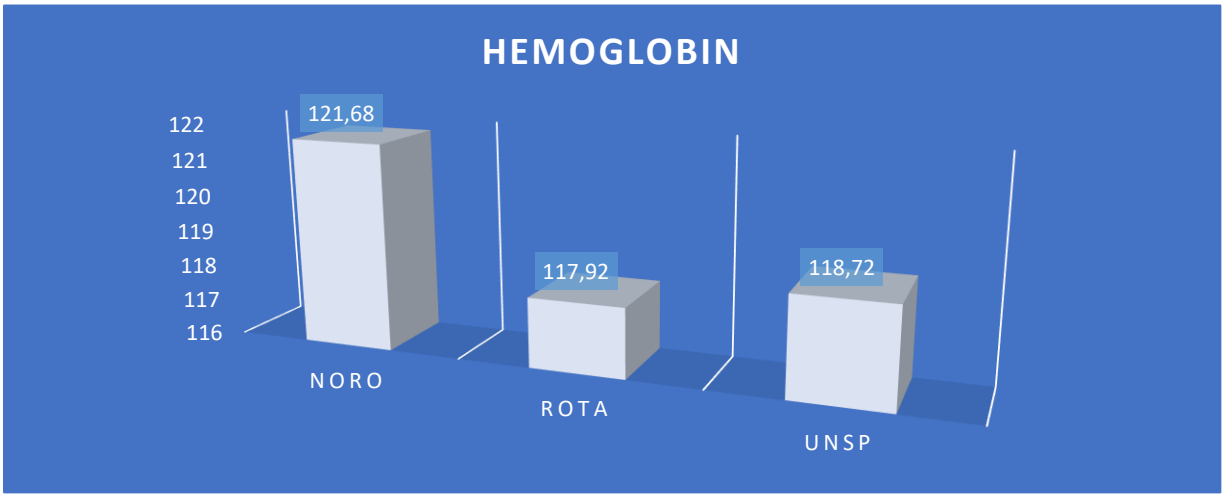


Fig. 28. Comparative analysis of the average values of hemoglobin by etiological agents:

Fig. 28 shows that the average values of hemoglobin in patients with rotavirus enteritis are almost the same as the average values of patients with unspecified viral intestinal infections, and they do not differ significantly from the average values of hemoglobin in norovirus infections

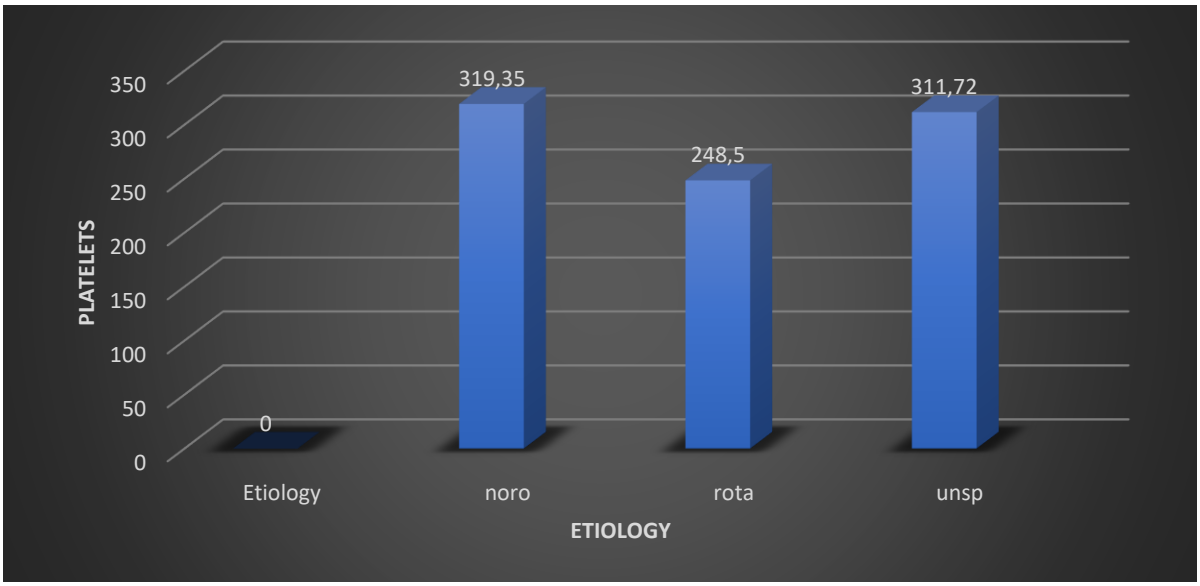


Fig. 29. Comparative analysis in the average values of platelets by etiological, compared agents

From fig. 29 it can be seen that the average value of platelets is the highest in patients with norovirus enteritis and the lowest in patients with rotaviruses, as is the ratio of leukocytes

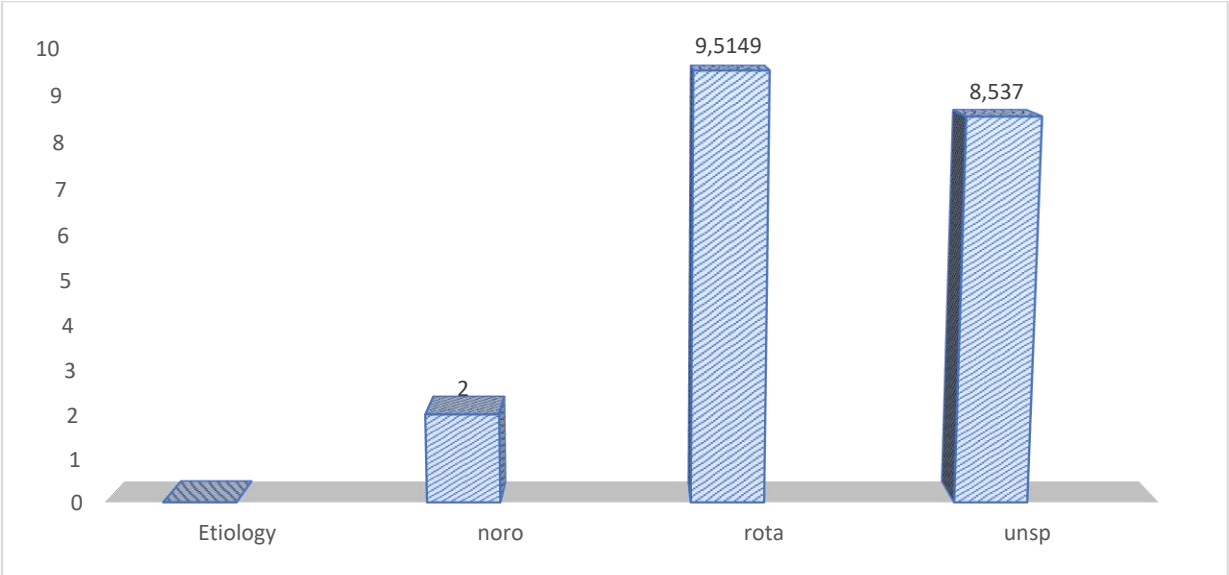


Fig. 30. Comparative analysis in the average values of SUE according to the etiological, compared agents:

Although Figure 30 clearly shows the difference in mean ESR values for rotaviruses and platelets, the data we have for norovirus enteritis is not sufficient to demonstrate a statistically significant difference between ESR values for the two viruses.

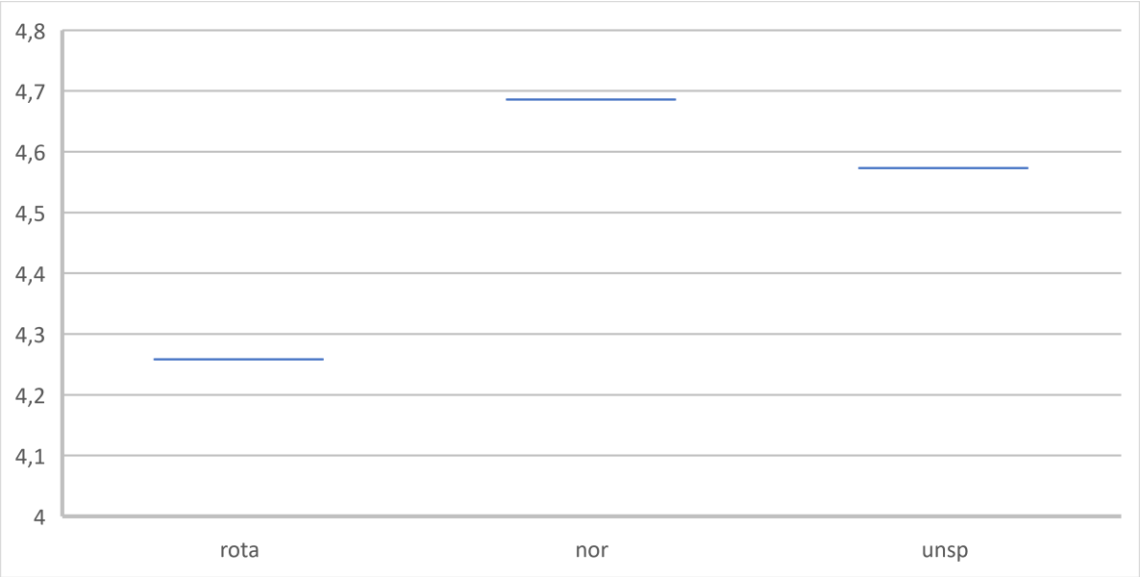


Fig.31.Comparative analysis of the average potassium (K) values among patients with rotavirus (rota), norovirus (noro), and unspecified viral gastrointestinal infections

From Figure 31, it can be observed that we have not established a statistically significant correlation regarding the potassium (K) trace element when comparing the etiological agents. The lowest average levels of K are found in patients with rotavirus enteritis, and within this group, we have also noted its lowest level.

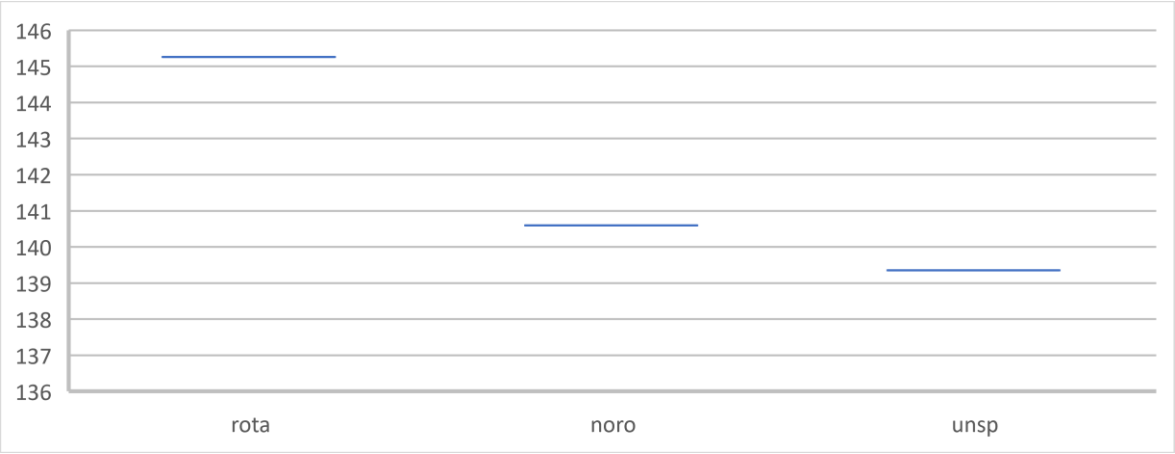


Fig.32.Comparative analysis of the average sodium (Na) values among patients with rotavirus (rota), norovirus (noro), and unspecified viral gastrointestinal infections

From Figure 32, it can be seen that we have not established a statistically significant relationship between the element Na (sodium) and the compared etiological agents. The lowest levels of Na are observed in patients with unspecified viral gastrointestinal infections.

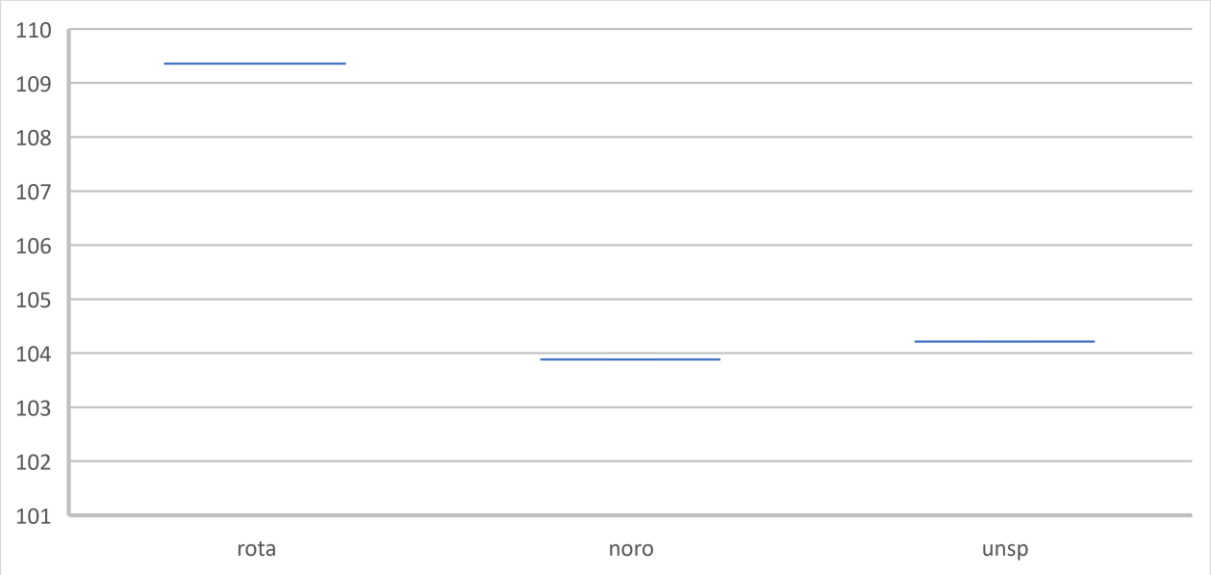


Fig.33.Comparative analysis of the average chlorine (Cl) values among patients with rotavirus (rota), norovirus (noro), and unspecified viral gastrointestinal infections

From Figure 33, it is evident that we have not established a statistically significant relationship between Cl (chlorine) and the compared etiological agents. The lowest levels of Cl are found in patients with norovirus enteritis.

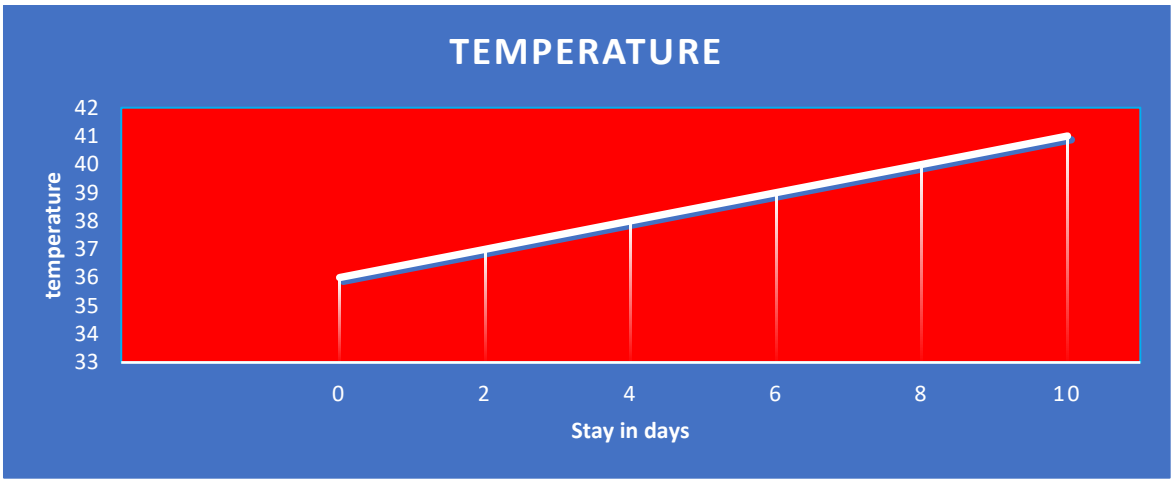


Fig. 34. Comparative analysis between values of febrility and duration of hospital stay

If above the rotavirus data by themselves did not show a statistically significant relationship between febrility and length of hospital stay, then the data for all viral intestinal infections taken together show that the higher the febrility, the longer the length of the hospital stay. the hospital stay, that is, the relationship is right.

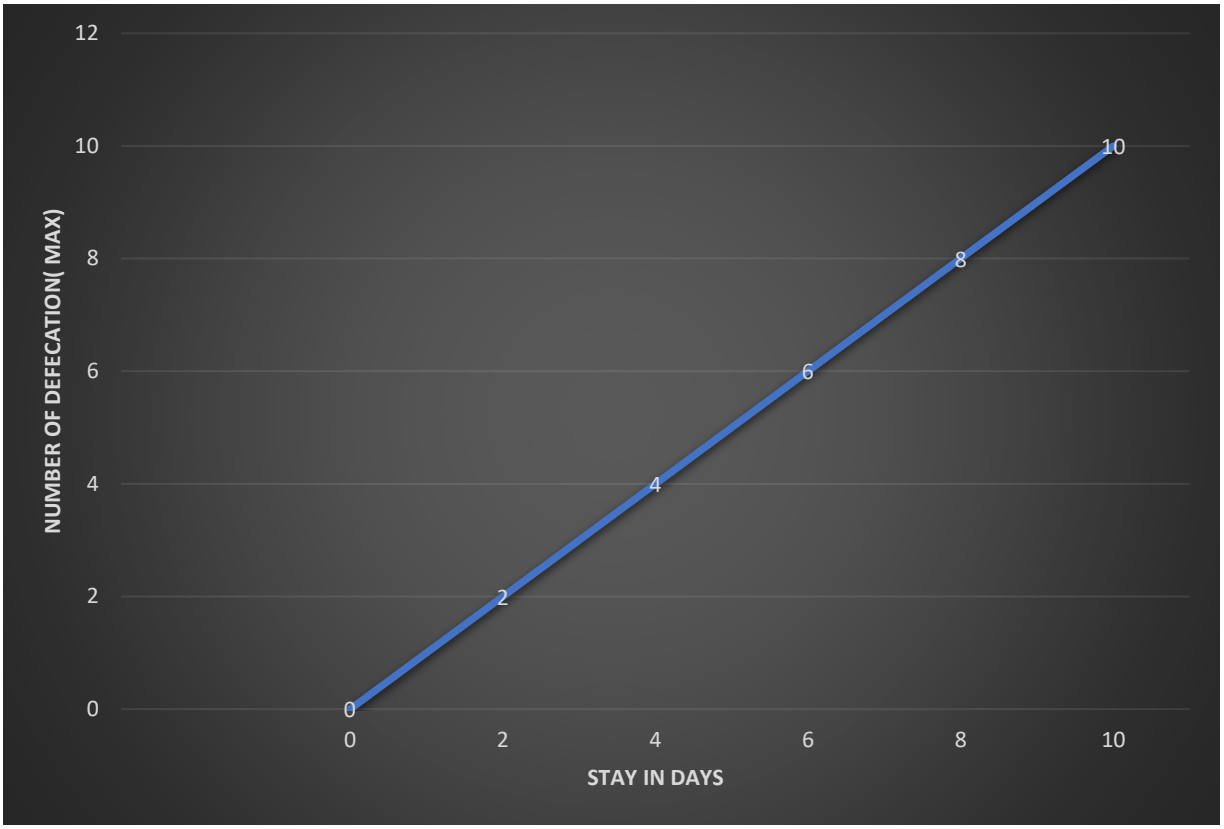
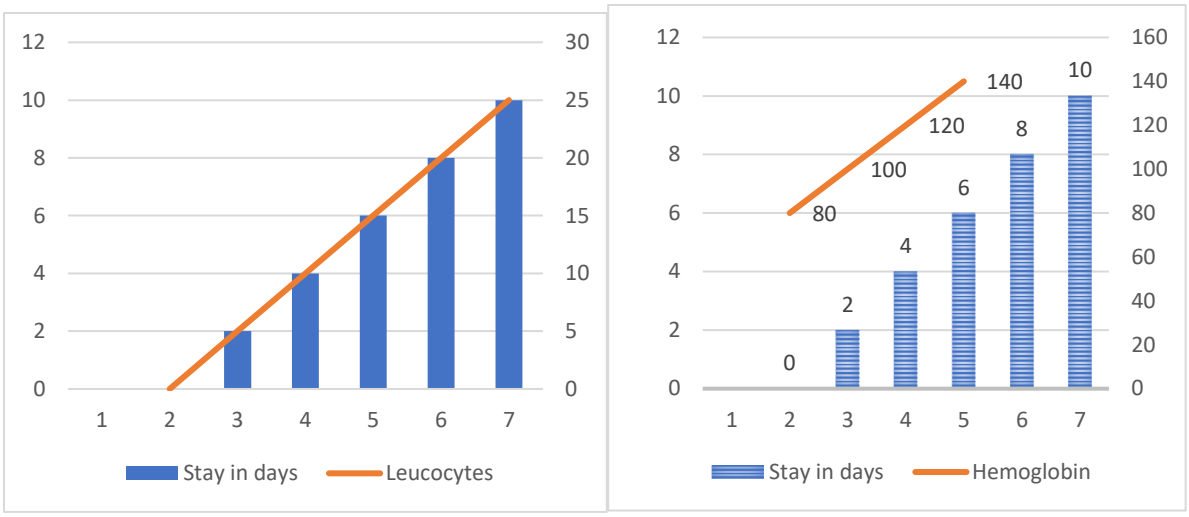
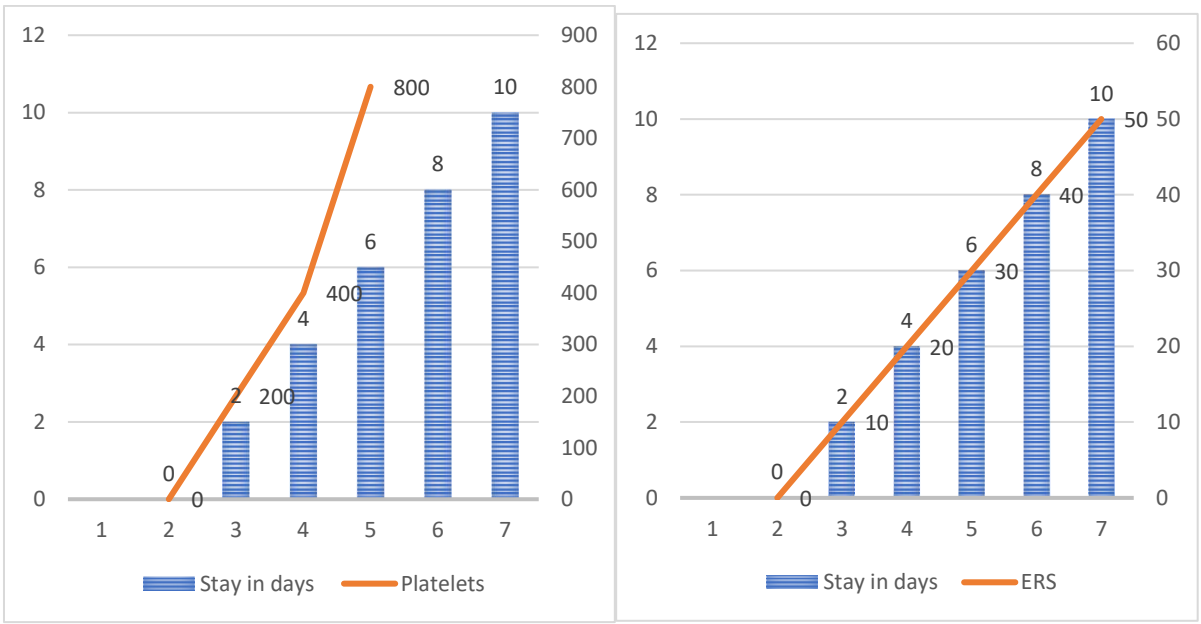


Fig.35. Comparative analysis of the length of hospital stay and the maximum number of defecations

From fig. 35 it is also clear that with an increase in the maximum number of defecations, in general for all examined patients, the duration of the hospital stay also increases.



a) b)



c) d)

Fig. 36 a, b, c, d. Comparative analysis of the length of hospital stay and the values of the main examined clinical and laboratory indicators in all examined patients: (a-leukocytes, b-hemoglobin, c-platelets, d- ESR)

Fig. 36 shows that no statistically significant relationship was established between the duration of the hospital stay and the main clinical and laboratory indicators in our patients.

Statistical relationships between viral intestinal specified infections:

There is a statistically significant difference in the average ages by ethnicity - $p=0.019<0.05$:

Patients of Bulgarian origin, who are a total of 128 of all with viral intestinal specified infections, have an average age of 2.56 years, and those of minority origin, who are a total of 17 – 1.01 years

There is a difference regarding diarrhea, which, however, is not statistically significant, since most of the children of Bulgarian origin (109 out of 128) also have diarrhea

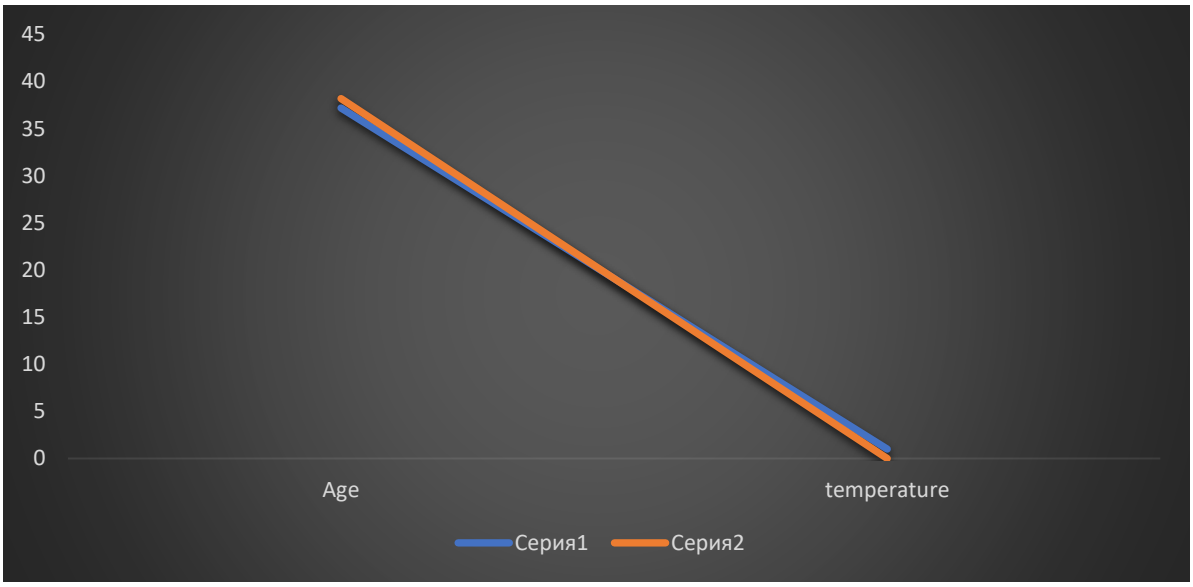


Fig. 37. Age and febrility

With a statistical level of significance - p=0.007<0.05 are the values of febrility in children under two years of age and in older children

Children up to two years inclusive are 88, with an average temperature of 38.238

Children over two years old are 57 with an average temperature of 37.721

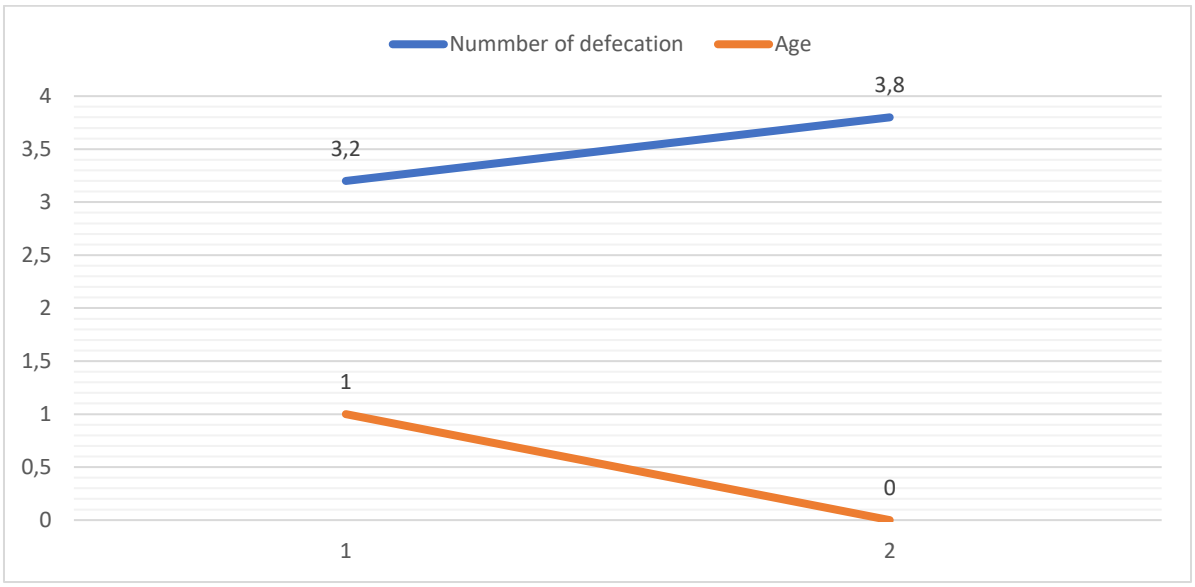


Fig. 38. Age and average number of bowel movements.

There are similar indicators for the number of defecations - older children have significantly fewer defecations -3.12 vs. 3.81, p=0.074 close to 0.05

There is also a significant difference in diarrhea - significantly fewer children here have diarrhoea, in the older ones - a mean value of 1.75 vs. 1.94 (average between 1 and 2 as a diarrhea category, reflecting the lower proportion in the older ones), p =0.001

It is also significant for vomiting - average value in adults of 1.81 versus 1.96 in children, p=0.006

There is also a significant difference in hemoglobin levels - mean value of the large 121.874 (43 patients) versus 117.209 for the small (86 patients), p=0.017 < 0.05

DISCUSSION:

In a prospective study involving 206 patients, a variety of etiological agents causing infectious gastroenteritis was observed. Viral intestinal infections, regardless of the causative agent, have similar clinical symptoms. Modern immunochromatographic tests are used to clarify their etiological diagnosis, and molecular biological methods are used for verification.

The largest percentage of etiologically confirmed intestinal infections are rotavirus, despite the availability of a vaccine in the country. Voluntary vaccinations in Poland have also not led to a significant decline in the prevalence of rotavirus infections, and they continue to be the leading etiology of viral gastroenteritis there as well, although vaccination coverage is estimated to be 25%. Viral intestinal unspecified infections are next, followed by noroviruses, and adenoviruses and astroviruses are proven extremely rarely

The data from our study are consistent with the data of authors from Nigeria who conducted a study in 2008, who also reported that rotavirus remains the most common cause of non-bacterial gastroenteritis, as well as with data from authors from India and Egypt who also indicated, that the main etiological agent even after 2019 they still have rotavirus, but with the difference that it is in first place before adeno and astroviruses, without comparison with norovirus gastroenteritis

Virological tests conducted in France in the period 1997-1999 detected rotavirus in 17.3% of samples, calicivirus in 7.3%, astrovirus in 6.8%, adenovirus in 0.7%, and data from 2002. in Germany indicate that rotavirus occurs in 79% (102/129), while norovirus in 35%, intestinal adenovirus in 14% and astrovirus in 4%.

Data from a study conducted in Brazil in 2006, after the introduction of rotavirus vaccination in the country, indicated that 16% of the examined patients had adenovirus, 8% norovirus, 6% rotavirus and 0% astrovirus. These data show that the introduction of rotavirus vaccination in the country significantly contributes to the reduction of hospitalizations from rotavirus enteritis. Data from the authors from Taiwan, even after the introduction of the rotavirus vaccine, showed that the frequency of confirmation of rotavirus infections was 21.2%, norovirus-14.9%, adenovirus-3.74 and astrovirus-2.10%.

Studies in Turkey prove that adenovirus is the leading cause of non-bacterial gastroenteritis in children. Authors from Japan, in a 2007-2008 study, found the same frequency of hospitalizations from noro- and rotavirus enteritis and concluded that the severity of hospitalizations from norovirus enteritis did not differ significantly from those with rotaviruses

The highest frequency of isolation of astroviruses from fecal samples was reported by authors from Northern Italy in 2018, and authors from Thailand reported a higher frequency of isolation of astroviruses compared to adenoviruses.

Our data show that with etiologically proven viruses, children in the age group of one to five years are mainly affected. A large proportion of children with unspecified viral intestinal infections tend to be over five years of age. In the age up to one year, there is no dominance of certain etiological agents, however, it is striking that adenoviruses and astroviruses, although in a small percentage, are observed more often in this age range.

Our analyzes coincide with the data of most authors from developed countries and Brazil.

The number of patients with rota- and norovirus infections in the age group from one to five years is the largest, as well as viral intestinal unspecified infections, although compared to the other etiologically specified agents, the relative share of children is the largest in viral intestinal unspecified infections in the age group 5-10 years. These data coincide with the data from the studies of Prof. Nelly Korsun, conducted in the period 2005-2009. and show that viral intestinal unspecified infections are mainly

observed in the older age groups. Bangladeshi researchers report that norovirus infections are more common in children under 24 months of age

We found a statistically significant difference in terms of gender in relation to the etiological agent in the compared groups. Male gender predominated in patients with rotavirus enteritis compared to patients with noroviruses and unspecified viral enteric infections ($p<0.05$) Luo L, Gu Y et al also found significantly higher levels of confidence intervals in patients with rotavirus enteritis in the younger age groups, establishing the dominance of the female gender in the older ones.

A higher frequency of distribution among males and in younger age groups is also proven by researchers from Iran. However, authors from Poland reported a higher incidence of rotavirus diarrhea in girls compared to bacterial gastroenteritis in boys.

Norovirus gastroenteritis in our study is more common in the winter-spring season, which coincides with the data of other Bulgarian authors. Authors from Germany reported an epidemic of norovirus gastroenteritis during the winter months, in the USA data show a high frequency of norovirus infections during the period October-May, in Bangladesh they circulate during the winter and rainy seasons, and data on the distribution of noroviruses in Latin America show that there they are found in all seasons, due to the great climatic differences in the region.

Adeno and astroviruses in our study were mainly observed in the autumn-winter months, which coincides with the authors' data from Mersin Hospital, Turkey.

We found a statistically significant difference in the average values of fever, with the highest average value reported in patients with rotavirus infections, and the lowest in patients with noroviruses. Authors from Brazil in studies on norovirus infections reported higher mean values of fever - over 38.2 degrees, which is in contrast to our study, however, other authors from Taiwan found higher values of fever in patients with rotavirus infections.

Vomiting and diarrhea were observed in all our patients with viral intestinal infections, both specified and unspecified, and we found statistically significant differences in the symptoms of abdominal pain and diarrhea with a significance level of $p<0.05$. Abdominal pain is much more common in patients with unspecified viral intestinal infections than in patients with rotavirus and norovirus enteritis, and diarrhea is absent in more than half of patients with norovirus gastroenteritis. The authors, who conducted a study in Brazil, reported that all their patients with norovirus gastroenteritis had a diarrheal syndrome. They even reported that 10.22% of their patients had blood in their stools.

We did not find a statistically significant difference in terms of the pathological impurities in the feces, which are mainly found among patients with rotavirus intestinal infections, but the number of patients is too small to be able to prove such a difference compared to the compared etiologic agents ($p>0.05$).

Respiratory symptoms were also observed in patients with viral enteric infections, and in our study they were in a greater percentage in patients with rotavirus enteric infections and unspecified viral enteric infections, but there was no statistically significant dependence in relation to them in relation to the etiological compared agents ($p>0.05$).

Our observations show a more severe course in rotavirus enteritis, with convulsive symptoms also present, but it occurs in a very low percentage of patients and we do not find a statistically significant difference between the compared etiological agents, just as we do not find such a difference in terms of the symptoms nausea and vomiting ($p>0.05$).

The authors from Taiwan reported a higher frequency of seizure symptoms in patients with norovirus infections, but because they had a larger number of patients, they also reported a statistically significant difference between the occurrence of seizure symptoms in patients with rotavirus infections, compared to those with norovirus enteritis ($p>0.05$).

Cases of norovirus were clinically indistinguishable from those of rotavirus origin in children under two years of age, while they were slightly milder in the older group of patients in a study done in Poland.

We also observe a statistically significant difference in two of the main clinical and laboratory indicators - leukocytes and platelets compared to the etiological agents. Leukocytes and platelets were significantly higher in patients with norovirus infections compared to those with rotavirus and unspecified viral intestinal infections ($p<0.05$). We did not find a statistically significant difference in terms of hemoglobin and erythrocyte sedimentation rate (ESR) in relation to etiologic agents ($p>0.05$).

We found a statistically significant difference between length of hospital stay by etiological agents.

The mean hospital stay for patients with rotavirus enteritis was 5.4118 days with a standard deviation of 1.70237, while for patients with norovirus enteritis it was 2.4545 days with a standard deviation of 1.60255 and for patients with unspecified viral intestinal infections 3.6909 days with standard deviation 1.03410 ($p<0.05$).

The hospital stay was the longest in patients with rotavirus enteritis, and the shortest in patients with norovirus enteritis. These data of ours differ from those of the authors from France, who reported that the mean duration of hospitalization was statistically significantly lower for children with rotavirus gastroenteritis despite more frequent dehydration observed among children with rotavirus compared to those with astrovirus or calicivirus gastroenteritis.

We also observe a statistically significant difference in the maximum number of defecations depending on the etiological agent ($p<0.05$).

We also did not find a statistically significant difference in terms of the length of hospital stay compared to the main clinical and laboratory indicators in all patients in our study ($p<0.05$).

Statistically significant reliable differences are found in the following categories of signs in patients with specified viral intestinal infections: the average ages in children of Bulgarian and minority origin, the average values of fever in older and younger children, vomiting in older and younger children. small children and diarrhea in older and younger children. For all these indicators listed above ($p<0.05$).

CONCLUSIONS:

1. In the presented prospective study of the followed hospitalized patients with viral intestinal infections since 2018. until 2022 at the age of zero to ten years, the relative share of patients in the age group of one to five years is the largest.

2. The male gender is predominant in the patients with rotavirus enteritis, while in the other compared groups we did not find a statistically significant difference regarding it ($p<0.05$).

3. The average value of febrility is the highest in patients with rotavirus enteritis compared to the other compared agents ($p<0.05$).

4. Convulsive symptoms and pathological impurities in the feces are observed in a very small part of patients with rotavirus enteritis, as well as respiratory symptoms. In a minor percentage, we register convulsive symptoms in patients with adenovirus enteritis and respiratory symptoms in patients with norovirus enteritis.

5. No predominance of the symptoms of nausea and vomiting was reported in the patients with different etiological agents.

6. Abdominal pain is more often demonstrated in patients with unspecified viral intestinal infections, which also include older age groups.

7. A significant percentage of patients with norovirus gastroenteritis do not have diarrhea syndrome.

8. A statistically significant difference was found in the values of platelets and leukocytes in rota- and norovirus gastroenteritis.

9. The longest average hospital stay was reported in children with rotavirus enteritis, and the shortest in those with norovirus gastroenteritis

CONTRIBUTIONS:

Contributions of an original scientific nature

1. A prospective analysis of circulating viral enteric infections over a four-year period was performed using immunochromatographic and high-sensitivity molecular methods.

2. The viral causes of diarrheal diseases in children up to 10 years of age were analyzed for a four-year period in two regions of the country

3. The leading role of rotavirus enteritis among other enteric viruses for the severe course of morbidity in patients under five years of age, regardless of the available vaccine, has been determined. The vaccine is currently recommended in Bulgaria. Vaccination coverage is extremely low and the vaccine could be included in the compulsory immunization calendar of the Republic of Bulgaria.

4. A comparative analysis was made regarding etiology, clinic and paraclinic and etiologically unproven intestinal viral infections during seasonal morbidity, covering a period of four years.

Scientific-applied and confirmatory contributions

1. Differences in the course of different intestinal infections by etiological agents, as well as viral intestinal infections with an unproven etiological agent, have been established.

2. The average hospital stay was evaluated depending on the type of intestinal viruses examined.

3.Etiologically confirmed and etiologically unconfirmed intestinal infections were compared in terms of age, gender, clinical signs and paraclinical indicators.

4. The sensitivity and specificity of the immunochromatographic tests used at the patient's bedside were compared with the highly sensitive molecular biological methods

PUBLICATIONS AND SCIENTIFIC ANNOUNCEMENTS RELATED TO THE DISSERTATION

Publications

1.Valeri Velev, Maria Pavlova, Ekaterina Alexandrova, Ivan Ivanov, Metodi Popov. Campylobacter inf Biotechnology & Biotechnological Equipment Section in children and adults in Bulgaria: comparative characteristics and antimicrobial resistance. 2022/12/31 36,1;292-298

2. Popov M. Velev V. Adenovirus infections. Medinfo. 2022(2): 18-20

3. Popov methods. Viral intestinal infections. Medical Digest.2022 (6):130-134

Participation with reports in scientific congresses/conferences:

1. Popov M., Tomov T, L. Nikolaeva-Glomb, T. Chervenyakova. Clinical-diagnostic studies on viral gastroenteritis in childhood. XIII national annual conference on infectious diseases - Starosel 03-05.10.2019

2. PopovM., V.VelevA.Mangerov, L.NikolaevaGlomb. Modern pathogenetic mechanisms in enterovirus infections and a review of therapeutic strategies applied to their treatment. XI National Virtual Congress on Infectious Diseases - Sofia 08-10.10.2020.

3. M. Popov, M. Pavlova. E. Alexandrova, Ivan Ivanov, Adriana Angelova, Eli Hristozova, Ivan Lyutakov, Valeri Velev. Cl. difficile infection after those who have recovered from covid 19. HIV National Conference on Infectious Diseases - Plovdiv 07-09.10.2021