Scientific Research Publishing

American Journal of Molecular Biology, 2024, 14, 211-229
https://www.scirp.org/journal/ajmb
ISSN Online: 2161-6663
ISSN Print: 2161-6620

Rotavirus, Norovirus and Astrovirus in Children Aged 0 - 5 Years: Evolution of Prevalence over 10 Years (2013-2023) Following the Introduction of Rotavirus Vaccines in Burkina Faso

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How to cite this paper: Dakouo, D., Ouattara, A.K., Ouermi, D., Traore, M.A.E., Zohoncon, T.M., Sawadogo, M., Zigani, N.L., Tao, N.I., Traore, L., Ouedraogo, T.-W.C., Ouedraogo, R.A., Kande, A., Gamsonre, Z., Bado, P., Ilboudo, D.P., Yonli, A.T., Djigma, F.W. and Simpore, J. (2024) Rotavirus, Norovirus and Astrovirus in Children Aged 0 - 5 Years: Evolution of Prevalence over 10 Years (2013-2023) Following the Introduction of Rotavirus Vaccines in Burkina Faso. *American Journal of Molecular Biology*, **14**, 211-229.
https://doi.org/10.4236/ajmb.2024.144016

Received: July 24, 2024
Accepted: September 26, 2024
Published: September 29, 2024

Abstract

Rotaviruses, noroviruses, and astroviruses are responsible for gastroenteritis in children under 5 years old. The objective of our study was to estimate the evolution of prevalence of rotavirus, norovirus and astrovirus infections in children aged 0 to 5 years with gastroenteritis, after the introduction of rotavirus vaccines in Burkina Faso. This cross-sectional study was conducted between January and December 2023, collecting 100 stool samples from children with gastroenteritis at Saint Camille Hospital in Ouagadougou and the Charles De Gaulle University Paediatric Hospital. Noroviruses and astroviruses were detected using multiplex real-time PCR with a Sacace biotechnology detection kit. Data analysis was performed with Stata statistical software, version 16.0. The prevalence of norovirus infections was 14% and astrovirus infections were 9%. Rotavirus infections were found at prevalence of 15%. The age group most

affected by norovirus and astrovirus infections was 0 - 12 months, with respective prevalence rates of 73.34% and 55.56%. The most frequently observed clinical signs in children infected with astrovirus were fever (77.78%), diarrhea (55.56%), and vomiting (44.44%). The introduction of rotavirus vaccines has reduced rotavirus-related infections. However, this has not significantly impacted the prevalence of norovirus and astrovirus infections in Burkina Faso.

Keywords

Rotavirus, Norovirus, Astrovirus, Gastroenteritis, Rotavirus Vaccines, Burkina Faso

1. Introduction

Rotaviruses were first described in 1973 by Bishop *et al.* in duodenal biopsies from children with diarrhea [1]. They belong to the Reoviridae family and form the genus Rotavirus [2] [3]. The diameter of the rotavirus particle is approximately 70 nm, and its genome is approximately 18,500 bp [4] [5]. The genome is composed of 11 segments of double-stranded RNA (dsRNA) encoding six viral structural proteins (VPs) and six non-structural proteins (NSPs). Segments 1, 2, 3, 4, 6, and 9 encode the proteins VP1, VP2, VP3, VP4, VP6, and VP7, respectively. The NSP1, NSP2, NSP3, and NSP4 proteins are encoded by genes 5, 8, 7, and 10, respectively. Segment 11 encodes the NSP5 and NSP6 proteins [6].

Noroviruses were first detected by electron microscopy in the stools of children suffering from gastroenteritis in 1972 [7]. They belong to the Caliciviridae family and are non-enveloped viruses with single-stranded RNA of positive polarity [5]-[8]. The diameter of the norovirus particle varies between 25 and 40 nm, with a genome consisting of 7500 to 7700 bp [9] [10]. The norovirus genome is organized into three open reading frames (ORF-1, ORF-2, and ORF-3). ORF1 encodes non-structural proteins, ORF2 encodes the capsid protein VP1, and ORF3 encodes the minor structural protein VP2. The non-structural proteins include p48 (NS1/2 or N-term), NTPase (NS3 or 2C-like), p22 (NS4 or 3A-like), VPg, 3C-type protease (3CLpro or NS6 or 3C-like), and RNA-dependent RNA polymerase [9] [10]. The VP1 and VP2 proteins are structural proteins that encapsulate the viral genome [11]. Noroviruses are highly diverse. Based on the VP1 capsid, noroviruses are classified into 10 genogroups (GI-GX) and 49 genotypes [12]. The genogroups GI, GII, GIV, GVIII, and GIX infect humans, with GII being largely predominant [10] [12]-[14].

Astrovirus particles were identified in 1975 [2] [3]. They belong to the Astroviridae family and they are non-enveloped, single-stranded RNA viruses of positive polarity [5]-[7]. The particle diameter of the astrovirus ranges between 28 and 30 nm, with a genome size varying between 6400 and 7300 bp [8] [9]. The astrovirus genome contains three open reading frames (ORFs): ORF1a, ORF1b, and ORF2, which encode the RNA polymerase, RNA-dependent proteins, and the

structural protein of the virus, respectively [15].

Rotaviruses, noroviruses, and astroviruses are primarily transmitted through the fecal-oral route via contaminated water and food, person-to-person contact, and contaminated surfaces [16]-[20].

Airborne transmission through contaminated aerosols has also been reported [20]-[22].

Rotaviruses and noroviruses predominantly target mature epithelial cells of the intestinal villi in various species of mammals and birds [23]. Clinical manifestations of rotavirus and norovirus infections include diarrhea, vomiting, abdominal pain, fever, and dehydration [24]-[26]. Astrovirus infections also present with these symptoms, along with nausea [17]. Currently, there are no vaccines or antiviral medications available for noroviruses or astroviruses [17] [27]. However, four WHO-prequalified vaccines (Rotarix, RotaTeq, RotaSiil, and Rotavac) are available for the prevention of rotavirus infections [28] [29]. Despite the availability of these vaccines, there are no specific antiviral agents targeting rotavirus diseases [3].

Epidemiologically, rotaviruses, noroviruses, and astroviruses are significant contributors to gastroenteritis, imposing substantial morbidity and mortality worldwide [30]. Group A rotaviruses (RVA) are the most prevalent, accounting for over 90% of human rotavirus infections [2]. In 2013, rotavirus-related deaths in children under five years old in sub-Saharan Africa exceeded 120,000 [31]. The highest mortality rates due to rotavirus infections are in sub-Saharan Africa, Southeast Asia, and South Asia [32]. Norovirus infections affect individuals of all ages, with the highest hospitalization rates observed among children under 5 years old [10]. Globally, noroviruses cause nearly 200 million cases of diarrhea and lead to 50,000 deaths in children under 5 years old, predominantly in low- and middle-income countries [30]. Hospitalization rates for norovirus-related gastroenteritis in these regions are estimated at 17% and 12%, respectively [33]. Norovirus epidemiology varies across regions, with detection rates of 15% in Latin America, 17% in Asia (specifically China), and 19% in Africa among children under 5 years old with gastroenteritis [10] [33] [34].

Astroviruses, similar to rotaviruses and noroviruses, are found globally [17] [19]. They are often linked to sporadic outbreaks of diarrhea, particularly among children in hospitals, daycares, kindergartens, and schools [35]. Astroviruses account for about 10% of sporadic diarrhea cases in children, with detection rates ranging from 2% to 9% in children with acute diarrhea. In low-income countries, prevalences as high as 26% have been reported [35]. To address the burden of viral gastroenteritis, vaccines targeting rotaviruses have been introduced. These vaccines, recommended by the WHO in 2007 and reaffirmed in 2013, have led to a substantial reduction in rotavirus-related diarrhea among children under 5 years old [36]. Studies conducted after the introduction of rotavirus vaccines, such as in Burkina Faso, have shown a decline in the prevalence of rotaviruses, indicating their effectiveness [25].

Following the implementation of rotavirus vaccines, an increase in norovirus detection rates and a rising prevalence of severe norovirus-related gastroenteritis have been observed in some regions [37]-[39]. As rotavirus vaccines become more widespread, noroviruses may become the most common pathogens causing severe diarrhea. In Burkina Faso, the rotavirus vaccines Rotateq (introduced in 2013) and RotaSiil (introduced in 2019) have been implemented [29] [40]. Given the extensive use of rotavirus vaccines, it is crucial to assess their impact on the prevalence of rotaviruses, noroviruses, and astroviruses in the socio-economic context of Burkina Faso. However, limited studies have been conducted in this area, emphasizing the need to closely monitor the epidemiology of these infections post-vaccine introduction. This study aims to estimate the changes in prevalence and describe the clinical characteristics of norovirus and astrovirus infections among children aged 0 to 5 years with gastroenteritis.

2. Methodology

2.1. Ethical Considerations

The study adhered to the ethical principles outlined in the Declaration of Helsinki and received approval from the Institutional Review Boards of HOSCO, CHUP-CDG, and the Ethics Committee for Health Research of Burkina Faso. Prior to sample collection, all parents or guardians of the children provided free and informed consent. Measures were implemented to maintain the confidentiality of collected data and ensure patient anonymity. Specifically, codes were generated by the study sponsors to allow matching between patients and samples while preserving confidentiality.

2.2. Study Framework

The samples for this study were collected from two healthcare facilities in Ouagadougou, Burkina Faso: Saint Camille Hospital in Ouagadougou and Charles De Gaulle University Paediatric Hospital.

All laboratory work and technical procedures were conducted at the Laboratory of Molecular and Genetic Biology and Pietro Annigoni Biomolecular Research Centre. Laboratory of Molecular and Genetic Biology is a research laboratory affiliated with Doctoral School of Science and Technology of Joseph KI-ZERBO University. Established in 2007, Laboratory of Molecular and Genetic Biology's mission is to contribute to scientific advancement by training master's and doctoral students in applied molecular biology and genetics. Laboratory of Molecular and Genetic Biology was recognized as a Center of Excellence by the West African Economic and Monetary Union (WAEMU) in February 2015. Similarly, Pietro Annigoni Biomolecular Research Centre equipped with modern facilities, plays a pivotal role in training biologists, doctors, and pharmacists from Burkina Faso and the wider West African sub-region. Pietro Annigoni Biomolecular Research Centre also contributes to enhancing healthcare quality in Burkina Faso and West Africa and serves as a National Reference Laboratory for Human Papilloma Virus

(HPV).

2.3. Study Type and Population

This cross-sectional study was conducted between January and December 2023, focusing on children aged 0 to 5 years who were diagnosed with gastroenteritis and subsequently admitted to the laboratory for prescribed bacteriological analyses. A total of 100 stool samples were collected from these participants. Gastroenteritis cases were defined by the presence of either at least three liquid or semi-liquid stools within a 24-hour period or stools that were more frequent and abundant than usual. Demographic, socioeconomic, clinical data, and rotavirus vaccination history were collected using an individual data collection form. The rotavirus vaccination status of each child was assessed by referring to the vaccination record provided by the Ministry of Health and Public Hygiene of Burkina Faso. This vaccination card documented whether the child had received complete vaccination (three doses of rotavirus vaccine), partial vaccination (one or two doses), or no vaccination against rotavirus. Two rotavirus vaccines were introduced in Burkina Faso: Rotateq in 2013 and RotaSiil in 2019 [29] [40]. Stool samples were collected using sterile jars following standard laboratory examination procedures. Upon collection, the samples were aliquoted and then stored at −80˚C to maintain their integrity for subsequent molecular analyses.

2.4. Determination of the Nutritional Status of Children: Calculation of Z-Scores

The nutritional status of each child was assessed using anthropometric data, including weight, height, and age. Child growth standards established by the World Health Organization were used for classification purposes. Nutritional status was determined by calculating Z-scores or SD-scores (standard deviation scores) using specific indices. Z-scores were calculated based on the ratios of "weight/height" to assess wasting and "height/age" to assess stunting. The severity of nutritional status was categorized as normal, moderate, or severe based on interpretive values, which are detailed in Table 1.

Table 1. Values for assessing the nutritional status of children according to Z-socres.

Z-score value	Degree of nutritional status
$x < -3$	Severe malnutrition
$-2.99 < x < -2$	Moderate malnutrition
$x > -2$	Normal

Legend: the x value is the nutritional index calculated based on the "weight/height" (WHZ), "height/age" (HAZ) and "weight/age" (WAZ) ratios.

2.5. Real-time Multiplex PCR Detection of Rotaviruses, Noroviruses and Astroviruses

The collected samples were subjected to real-time RT-PCR. A Rotavirus/Norovirus/Astrovirus Real-TM kit from Sacace biotechnology was used for the detection of

rotavirus, genogroup 2 (GII) of norovirus and astrovirus in children's stools. For the molecular diagnosis of rotavirus, norovirus and astrovirus, the following steps were followed: preparation of stool samples, extraction of rotavirus, norovirus and astrovirus RNAs, reverse transcription of the extracted RNAs into complementary DNA (cDNA), and amplification of cDNAs by real-time PCR. Fecal suspensions (20%) were prepared by combining 4 ml of phosphate-buffered saline (PBS) with 1 g (or 1 ml) of stool in 5 ml Eppendorf tubes. The tubes were vortexed until thoroughly homogenized, followed by centrifugation at 10,000 g for 5 minutes. The resulting supernatants were then transferred to appropriate 1.5 ml tubes. To lyse the samples, 100 µl of the previously collected supernatants in the 1.5 ml tubes were added to 450 µl of the lysis solution and 10 µl of the internal control (Internal Control, IC RNA). For RNA fixation, 25 µl of an absorbent solution (Sorbent) containing silica particles was added. Impurities were removed through a washing step using 400 µl of washing solution. RNA precipitation was achieved by adding 500 µl of 70% ethanol in a repeated step. Proteins were then precipitated and removed by adding 400 µl of acetone.

Finally, the RNA was extracted from the silica particles using 50 µl of elution buffer (RNA-eluent).

Reverse transcription and amplification were performed in a total reaction volume of 25 µl, consisting of 15 µl of reaction mix and 10 µl of RNA from each sample. Amplification was conducted utilizing the SaCycler-96 Real-Time PCR System (Sacace Biotechnology), following the program specified in Table 2.

Table 2. Reverse transcription and amplification program.

Temperature	Time	Number of Cycles
50˚C (Reverse transcription)	30 min	1
95˚C (Initial denaturation and activation of Taq polymerase)	15 min	1
95˚C (Denaturation)	10 s	
60˚C (Hybridization)	35 s	45
72˚C (Elongation)	10 s	

The results were analyzed using the software of the SaCycler-96 Real-Time PCR System device with the "infections diseases" program. Analysis depended on the presence and position of the fluorescence curve relative to the threshold line (Ct) as shown in Table 3.

Table 3. PCR results interpretation window.

IC and norovirus were detected by the PCR-mix-Norovirus/IC.	The IC on the FAM channel (Green)	
	Norovirus on the JOE (Yellow)/HEX/Cy3 channel	
Rotavirus A and Astrovirus were detected with Rotavirus/Astrovirus PCR-mix-1.	Rotavirus A on the FAM channel (Green)	
	Astrovirus on the JOE (Yellow)/HEX/Cy3 channel	

PCR validation was confirmed if negative controls remained negative (indicating absence of exponential amplification), and when exponential amplification was observed for internal and positive controls with a Ct < 33. A sample was deemed positive if an exponential amplification curve was observed with a Ct < 33. Conversely, a sample was classified as negative if the result was positive only on the FAM channel with the PCR-mix-Norovirus/IC and the Ct value was < 33. Any result was considered invalid if the fluorescence curve intersected the threshold line but lacked the typical exponential growth phase.

2.6. Statistical Analyzes

Data analysis was conducted using Stata statistical software (version 16.0; Stata Corp., College Station, TX). Excel software was utilized to create frequency histograms. The chi-square (χ^2) test determined associations between variables (including socio-demographic, clinical, and nutritional data, as well as norovirus and astrovirus infections). A p-value ≤ 0.05 indicated statistical significance. Categorical variables were presented as frequencies, while continuous variables were expressed as means ± standard deviations or medians with interquartile ranges. Nutritional status was assessed by calculating Z-scores using WHO Anthro Survey Analyzer software, version.

3. Results

3.1. Socio-Demographic Characteristics of the Study Population

Out of the 100 stool samples collected, 55% (55/100) were obtained from male children, and 45% (45/100) were from female children. The mean age of the children was 21.65 ± 16.8 months. Additionally, the mean weight and height were 9.69 ± 3.92 kg and 81.54 ± 17.49 cm, respectively. The distribution of the study population across different age groups is presented in Table 4.

Table 4. Mean age, weight and height of children according to age groups.

Age range	Frequency n (%)	Mean age (month)	Mean weight (kg)
0_12	40% (40/100)	7.06 ± 2.78	6.76 ± 1.38
13_24	31% (31/100)	19.39 ± 4.01	9.20 ± 1.74
25_60	29% (29/100)	44.17 ± 11.61	14.26 ± 3.78

Among the study population, 13% of children were stunted, and 30% were underweight. Severe and moderate malnutrition were observed in 18% and 19% of children, respectively. Table 5 illustrates the distribution of the study population based on anthropometric parameters.

Table 5. Distribution of the study population according to nutritional status and by age groups.

Age groups (months)	N (%)		WHZ n (%)	HAZ n (%)	WAZ n (%)
0 - 12		x < −3	8 (20)	0 (00)	5 (12,50)
		−2.99 < x < −2	7 (17,5)	3 (7,50)	8 (20)
		x > −2	25 (62,50)	37 (92,50)	27 (67,50)

Continued

p-value		0,630	0,268	0,212
	x < −3	9 (29,03)	2 (6,45)	3 (9,68)
13 - 24	−2.99 < x < −2	4 (12,90)	5 (16,13)	9 (29,03)
	x > −2	18 (58,06)	24 (77,42)	19 (61,29)
p-value		0.134	0.114	0.001
	x < −3	1 (3,45)	2 (6,90)	1 (3,45)
25 - 60	−2.99 < x < −2	8 (27,59)	1 (3,45)	4 (13,79)
	x > −2	20 (68,97)	26 (89,66)	24 (82,76)
p-value		<0.001	<0.001	<0.001

3.2. Compensation for Children According to the Number of Doses of Rotavirus Vaccines Received

More than half 62% (62/100) of children had received the full course of three doses of rotavirus vaccine. The rates of children who received one and two doses of the vaccine were 11% (11/100) and 27% (27/100), respectively.

3.3. Prevalence's of Rotavirus, Norovirus and Astrovirus in Children with Gastroenteritis

Out of a total of 100 samples tested from children with gastroenteritis, at least one virus was detected in 38% (38/100) of cases. The prevalence's of rotavirus, norovirus and astrovirus were 15% (15/100), 14% (14/100) and 9% (9/100), respectively.

3.4. Distribution of Rotavirus, Norovirus and Astrovirus Infections by Age Group

The children most affected by rotavirus infections were those aged 0 - 12 months and 13 - 24 months with respective prevalence's of 73.34% (11/15) and 20% (3/15). Among children infected with norovirus, the age group of 0 - 12 months was the most affected with a prevalence of 64.29% (9/14), followed by that of 13 - 24 months with a rate of 28.57% (4/14). Children aged 25 - 60 months were the least affected with a prevalence of 7.14% (1/14). Among the three age groups, the detection rate of astroviruses was highest in children aged 0 - 12 months, 55.56% (5/9) (Figure 1).

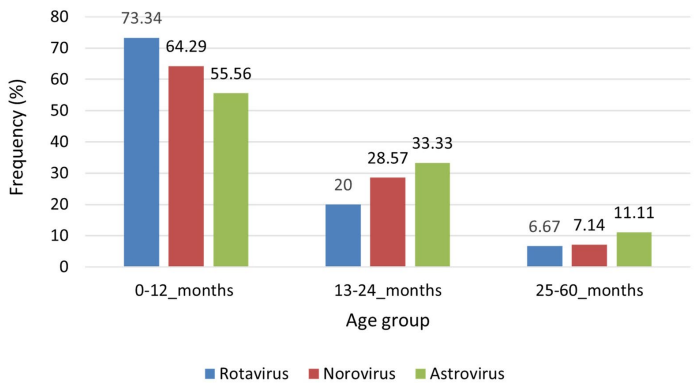

Figure 1. Distribution of children testing positive for rotavirus, norovirus and astrovirus according to age groups.

3.5. Clinical Manifestations of Rotavirus, Norovirus and Astrovirus Infections in Children

Diarrhea, vomiting, and fever were the predominant clinical manifestations in cases of rotavirus infections, with prevalence's of 60%, 73.33%, and 53.33%, respectively (Table 6). In children infected with norovirus, the most frequently observed clinical signs were diarrhea, fever, and vomiting, with prevalence's of 85.71%, 64.29%, and 35.71%, respectively. For astrovirus infections, the most frequent clinical manifestations were fever (77.78%), diarrhea (55.56%), and vomiting (44.44%).

Table 6. Distribution of children testing positive for rotavirus, norovirus and astrovirus based on clinical signs.

Symptoms		Rotavirus positive	Frequency (%)	p-value	Norovirus positive	Frequency (%)	p-value	Astrovirus positive	Frequency (%)	p-value
Diarrhoea	Yes	9	60 (9/15)	0.001	12	85.71 (12/14)	0.221	5	55.56 (5/9)	0.254
	No	6	40 (6/15)		2	14.29 (2/14)		4	44.44 (4/9)	
Vomiting	Yes	11	73.33 (11/15)	0.001	5	35.71 (5/14)	0.915	4	44.44 (4/9)	0.631
	No	4	26.67 (4/15)		9	64.29 (9/14)		5	55.56 (5/9)	
Fever	Yes	8	53.33 (8/15)	0.001	9	64.29 (9/14)	0.221	7	77.78 (7/9)	0.071
	No	7	46.67 (7/15)		5	35.71 (5/14)		2	22.22 (2/9)	
Abdominal pain	Yes	4	26.67 (4/15)	0.446	3	21.43 (3/14)	0.255	1	11.11 (1/9)	0.118
	No	11	73.33 (11/15)		11	78.57 (11/14)		8	88.89 (8/9)	

3.6. Age and Clinical Manifestations of Rotavirus, Norovirus and Astrovirus Infections

Clinical manifestations linked to infections affected children aged 0 - 12 months the most, with prevalence's of 66.67% for diarrhoea and 91% for vomiting. In children aged 0 - 12 months infected with norovirus, vomiting was the most common clinical manifestation, with a prevalence of 80% (4/5). In children aged 13 - 24 months and 25 - 60 months, fever was the most frequently reported clinical sign, with prevalence's of 33.33% (3/9) and 11.11% (1/9), respectively. Astrovirus infections manifested more frequently in children aged 0 - 12 months, 13 - 24 months, and 25 - 60 months, with diarrhoea, vomiting, and abdominal cramps at rates of 60% (3/5), 50% (2/4), and 100% (1/1), respectively. The distribution of children affected by rotavirus, norovirus and astrovirus infections according to age groups and clinical signs is illustrated in Figure 2.

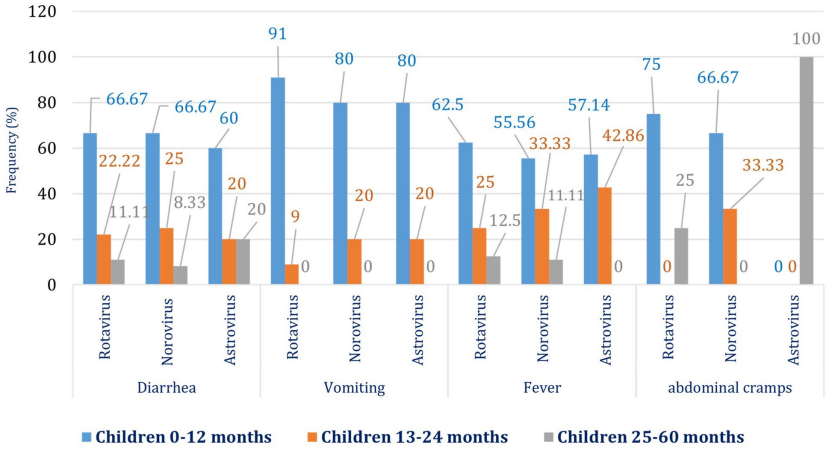

Figure 2. Distribution of children testing positive for rotavirus, norovirus and astrovirus according to clinical signs and age.

4. Discussion

The present study estimated the evolution of the prevalence of rotavirus, norovirus, and astrovirus infections after the introduction of rotavirus vaccines in Burkina Faso. In this study, rotavirus infections were recorded with a prevalence of 15%. This prevalence is significantly lower than those reported in previous studies conducted in Burkina Faso before the introduction of rotavirus vaccines, which recorded prevalence's of 21.10% [41], 30% [42], 32.40% [43], 44% [44] and 63.5% [40]. Following the universal use of rotavirus vaccines, a significant decrease in rotavirus-related illnesses and hospitalizations has been reported [45-47]. The prevalence of rotavirus observed in our study is similar to those reported in other studies conducted in several middle- and low-income countries after the introduction of rotavirus vaccines: 16% in El Salvador [48], 13.80% in Nigeria [49], 17.40% in Brazil [47], 18,50% in Venezuela [50] and 14.50% in Kenya [51]. The drop in the prevalence of rotavirus infections observed in our study could be explained by the substantial impact of the rotavirus vaccines introduced in Burkina Faso's expanded national vaccination program in 2013. This decrease in prevalence may be attributable to herd immunity, resulting from the overall protective effect of the vaccination program in vaccinated children in Burkina Faso. As supported by other studies, rotavirus vaccines are expected to provide long-term benefits once an expanded vaccination program is implemented [45] [52]. This study recorded a decline in rotavirus infections among children aged 0 - 5 years after the introduction of rotavirus vaccines in Burkina Faso. However, despite the introduction of these vaccines, rotavirus, along with norovirus, remains one of the leading viral pathogens causing gastroenteritis in children aged 0 - 5 years. The prevalence of norovirus observed in the present study was 14%. This prevalence is comparable to other studies conducted in Malawi and Ethiopia, which reported norovirus prevalence's of 12.1% [53] and 13.33% [54] respectively, after the introduction of rotavirus vaccines in these countries. However, in other low- and middle-income countries, higher prevalence's than those observed in the

present study have been reported after the introduction of rotavirus vaccines: 17.01% in Morocco [55], 16% in South Africa [56], 13.3% in Ethiopia [54], and 9.3% in Botswana [57] and Brazil [58]. Before the introduction of rotavirus vaccines, similar prevalence's were observed in several parts of the world: 15% in Brazil [58], 12% in Burkina Faso [59], 11.3% in Malawi [60] and 13.2% in Senegal [61].

The prevalence of astroviruses observed in the present study was 9% after the introduction of rotavirus vaccines in Burkina Faso. Before the introduction of rotavirus vaccines, the prevalence of astroviruses in Burkina Faso was 14.6% [62] and 4.9% [40] have been reported.

The prevalence of noroviruses and astroviruses observed in the present study highlights that norovirus remain important pathogens of gastroenteritis in children aged 0 - 5 years even after the widespread use of rotavirus vaccines. The prevalence of astroviruses observed in this study suggests their contribution to gastroenteritis in this age group, indicating a significant level of astrovirus carriage among children with gastroenteritis and potentially facilitating their transmission in the community. The observed prevalence of noroviruses and astroviruses in this study indicate that rotavirus vaccination did not have a positive impact on infections associated with these viruses.

In the present study, all enrolled children had received at least one dose of the rotavirus vaccine. Most children (89%) had received at least two doses of the rotavirus vaccine, with 27% having received 2 doses and 62% having received 3 doses out of the three that constitute the complete vaccination schedule. This result is similar to the rate of 87.7% reported by Suarez-Castaneda *et al*. for children who have received 2 doses [63]. But lower than the 78% reported by Gastanaduy *et al*. [64] for taking 2 doses. Additionally, 11% of children had received only one dose of the rotavirus vaccine, a prevalence similar to that reported by Platts-Mills *et al*. of 11.1% for one dose [53]. The situation of children who have not been able to complete the full course of rotavirus vaccination could be linked to vaccine shortages and missed opportunities. This observation highlights the importance of optimizing the performance of rotavirus vaccination in Burkina Faso. As supported by other studies, reducing the requirement for taking rotavirus vaccines linked to age restrictions would help achieve better vaccination coverage [63].

The present study revealed that a significant proportion (73.34%) of rotavirus-related infections were found in children aged 0 - 12 months. This age group was followed by the 13 - 24 month age group, with a prevalence of 20%. Combined, children aged 0 - 24 months accounted for 93.34% of rotavirus infections, indicating that this age group is the most affected. This finding aligns with a study in Burkina Faso that reported a prevalence of 92.9% for rotavirus infections in children under 2 years old [65].

Norovirus infections were detected across all age groups, and a statistically significant association was found between age and norovirus infections ($p = 0.0237$). The most affected age group was 0 - 12 months (0.29%), followed by 13 - 24

months (28.57%), resulting in a cumulative rate of 92.86% among infants aged 0 - 24 months. This finding aligns with the results of Eamonn *et al*. [60], who reported a predominance of norovirus infections in one-year-old infants. These results are also consistent with other studies that reported maximum detection rates for norovirus infections among children aged 4 - 23 months [66] [67]. For astrovirus infections, children aged 0 - 12 and 13 - 24 months were the most affected, with respective prevalence of 55.56% and 33.33%. The cumulative rate of 88.89% observed in children aged 0 - 24 months is slightly lower than that reported in a study conducted in Burkina Faso, which reported a prevalence of 92.30% in this age group [40].

The predominance of rotavirus (93.34%), norovirus (92.86%) and astrovirus (88.89%), infections in infants aged 0 - 24 months suggests greater community exposure during the child's first two years of life. This predominance could be attributed to the child's greater susceptibility to infectious agents during this age group.

The respective prevalence of 73.34%, 0.29%, and 55.56% for rotavirus, norovirus, and astrovirus observed in children aged 0 - 12 months indicate a higher susceptibility to infections associated with these viruses during the first 12 months of life. The lower detection of these viruses in children over one-year-old compared to younger children could be explained by the antibodies synthesized following previous infections during the first 12 months of life, providing some level of protection. In the case of rotavirus infections, the high prevalence among children aged 0 - 12 months could also be explained by a lower level of protection from rotavirus vaccines in low-income areas [28]. However, it has been reported that certain previous enteric infections induce interferences which can hinder the level of effectiveness of oral vaccines [68]. Infections linked to enteric pathogens are very common in low-income countries. In-depth studies on the lower level of efficacy of rotavirus vaccines in low-income countries are necessary, as the full reasons are not yet known. This research is needed to improve the level of efficacy of rotavirus vaccines, so that they are more effective and efficient where they are most needed.

The lowest detection rates for rotavirus (6.67%), norovirus (7.14%), and astrovirus (11.11%) were observed in children aged 25 - 60 months, which aligns with similar observations reported in another study [40]. This progressive decrease in infection rates for rotavirus, norovirus, and astrovirus suggests that the antibodies induced by previous infections with these viruses provide more optimal protection beyond the second year of a child's life.

The clinical manifestations linked to rotavirus, norovirus, and astrovirus infections in children were diarrhea, vomiting, fever, and abdominal pain. The prevalence rates of these clinical manifestations were as follows: for norovirus disease, they were 85.71% for diarrhea, 35.71% for vomiting, 64.29% for fever, and 21.43% for abdominal pain, as noted in studies [24] [25] [69] [70]. For astrovirus disease, the respective prevalence's were 55.56% for diarrhea, 44.44% for vomiting, 77.78% for fever, and 88.89% for abdominal pain, as reported in studies [40] [71]. Regarding rotavirus infections, the prevalence of diarrhea, vomiting, and fever were 60%,

73.33%, and 53.33%, respectively, consistent with observations in other studies [49] [72]-[74]. The predominant clinical signs of norovirus and astrovirus diseases were diarrhea, fever, and vomiting, respectively. It's worth noting that vomiting has been reported to occur more frequently than diarrhea [75]. However, there is currently no clear explanation regarding the predominance of one or the other of these three clinical signs.

Our analysis showed that the rates of clinical manifestations were higher in children aged 0 - 12 months (**Figure 1**). This finding suggests that children in this age group are more susceptible to norovirus or astrovirus illnesses. This susceptibility could be attributed to the child's relatively naive immune system during the first year of life. At this age, the immune system is fragile due to limited diversity of antibodies. Similar conclusions have been reported in this regard [76].

Limitations of the study The Rotavirus/Norovirus/Astrovirus Real-TM kit from Sacace biotechnology is a kit that only detects norovirus genogroup II. This could lead to an underestimate of the prevalence of noroviruses reported by this work.

5. Conclusion

Rotaviruses and noroviruses continue to pose significant health challenges, especially among children aged 0 - 5 years in Burkina Faso, despite the introduction of vaccines. These viruses remain at the forefront of causing gastroenteritis in this age group. The findings of this study align with global research indicating that norovirus genogroup II is a primary cause of gastroenteritis in children under 5 years old. Efforts to monitor and control infections caused by rotavirus, norovirus and astrovirus particularly in combating antibiotic resistance, should be intensified due to their significant impact on public health.

Authors' Contributions

JS, DO and DD designed the study; DD, N LZ, M S, and TM Z collected the samples; MAET, DD, MS, NLZ, CTWO ATO and AK carried out the technical manipulations; DD, MS and NLZ processed and analysed the data; DD prepared the manuscript; JS, DOI, AKO, NIT, TMZ, DI and LT revised the manuscript; JS, DO and A KO worked for the publication of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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