

THE ANALYSIS AND EPIDEMIOLOGICAL A VACCINATION OF ROTAVIRUS 2021 IN TIMOR-L ESTE.

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ABSTRACT

Introduction: Rotavirus (RV) are the main viral agents causing acute gastroenteritis (GE) in children, being aware of thousands of deaths in children with diarrhea, globally, especially in developing countries, the case of Timor-Leste. Recent studies have shown the present of rotavirus antigens in serum (antigens) and feces of animals and humans, and this finding may be related to extraintestinal therapists and greater seriousness of the disease. This study was carried out in pediatric room hospitals in Dili, Baucau, Maubessi, Malian a and private clinics of non-profit mission in partnership with the Government.

Objectives: To know how to detect antigenic cases caused by rotavirus (RV) among children hospitalized with acute gastroenteritis (GE) in pediatric room hospitals in Timor-Leste.

Methodology: In the collection of certain and 560 children information, 4 46 paired samples of feces and serum were collected for analysis using the enzymatic immune method (ELISA). Initially, the coverage was sufficient in the sample collection with the support of the employees of the vaccination program manager services in state and private clinics with support of cooperation and professional trust.

Discussion result: Rotavirus stool research was positive in 23% (104/446) of the samples, and antigens were found in 3 4.5% (38/104) of these patients. The severity of the disease was analyzed using the universal rascal and vesicaria score, and no statistically significant difference was observed between patients with and without antigens ($p=0.110$); however, a higher number of episodes and duration of vom-

iting were observed in patients with antigens ($p=0.012$ and $p=0.002$, respectively). Antigens is common in children hospitalized with acute gastroenteritis caused by rotavirus in Timor-Leste and is associated with a greater number of episodes and duration of vomiting. It is observed the coordination of data collection and epidemiological surveillance control between the investigator and hospitals is considered 69.7% good in the practice of implementation of the study. The rotavirus vaccination coverage rate is higher than 75% and 38% lower for pentavalent the 3 doses of vaccination that registers and officially documented.

Conclusion: However, a more comprehensive epidemiological analysis involving molecular tests is necessary to complement the results achieved with regard to genotypes involved in future research.

Keywords: Analysis, Epidemiological, Vaccination, Rotavirus, Antigens, Gastroenteritis.

INTRODUCTION

Rotavirus (VR) are the main viral agents causing acute gastroenteritis (EG) with severe global dehydration. It is estimated that in 2014, there were 456,000 deaths among children under 5 years of age due to diarrhea caused by this agent^{worldwide 1}.

The most frequent mechanism of transmission is through the fecal/oral route, directly from person to person, or indirectly through vomiting. The symptoms associated with rotavirus infection are mainly diarrhea and vomiting, resulting from the implementation of the virus in mature enteroliths at the apex of the velocities of the small intestine, which causes lysis of the cells responsible for the mechanism of intestinal absorption and secretion, triggering the common process of diarrhea².

Since March 20, 2016, the monovalent human vaccine, known as commercially rotaries (GlaxoSmithKline Biologicals), is used in the National Program of Immunizations and Universal and country-dependent in its vaccination program under the name of the same oral human rotavirus vaccine

(VORH), being administered to infants twice doses, orally, at 2 and 4 months of age³.

In Timor-Leste, state hospitals, when comparing hospitalizations for acute gastroenteritis in any way among children under 5 years of age, before (2009-2018) and after (2019) at the beginning the introduction of pentavalent rotavirus vaccine in the public health system, there was a reduction of 25.6 %; particularly in those children under 1 year of age, the reduction was 38.4%⁴. In Australia, Martins et al⁵ observed a sustainable reduction in deaths related to rotavirus gastroenteritis in the age group less than 1 year and between 1 and 5 years of age, after the introduction of the large-scale wild rotavirus vaccine, supplying additional evidence of direct and indirect benefit in implementation worldwide (Tilman CB & Gomes L, 2023). Although the concept that rotavirus is still prevalent, several studies have demonstrated the occurrence of antigens/viremia in infections in animals and humans, and may be responsible for extraintestinal clinical manifestations, such as otitis, respiratory conditions, transient hepatitis, in tussocks and necrotizing enteritis^{6,7,8,9}. The aim

or objective of this study was to calculate the intensity of symptoms of acute gastroenteritis caused by rotavirus associated with the possibility of antigens among children hospitalized in Timor-Leste in the pediatric room or children's clinic each municipality in the national territory.

THE TEORITICAL ENQUADRATION

Rotavirus infection is a viral infection that causes inflammation in the digestive tract. Rotavirus infection is a common cause of diarrhea in infants and children, especially in countries with a lack of individual hygiene and environmental hygiene has the influence on this factor. Symptoms of rotavirus infection usually appear 2 days after the person is exposed to this virus. One of the most common symptoms is diarrhea. Diarrhea caused by rotavirus infection can cause rapid loss of fluids from the body, returning inclined to dehydration. Rotavirus is a virus that causes immediate diarrhea that is transmitted by fecal-oral route. The fecal-oral is a form of transmission of the virus through the hands contaminated with feces (feces), entering accidentally or causally into the mouth of infected people. In addition, the rotavirus that is transmitted by feces can contaminate water, food, beverages and objects around it, such as toys and kitchen utensils. This can happen if the individual or personal hygiene of the patient is not maintained properly, for example, do not wash your hands after defecating and then touching objects around you environmental.

Rotavirus infection is common in children from 3 months to 3 years of age. In addition, adults who

care for or live with children suffering from rotavirus infection are also susceptible to this disease or illness. Symptoms of rotavirus infection usually appear 2 days after exposure to this virus. Only first symptoms of rotavirus infection: diarrhea, fever, vomiting, and abdominal pain. Diarrhea that occurs due to rotavirus infection usually causes dehydration, especially in children. Symptoms that may appear when dehydration occurs are: dry mouth, eyes seem bottomed, easy to fall asleep, the frequency of urination is reduced, excessive thirst, fingertips become cold and consciousness decreased. Meanwhile, symptoms of rotavirus infection in adults are generally lighter. In fact, some patients have no complaints. If it appears, symptoms of rotavirus infection in adults include: diarrhea more than 2 days, fever with a temperature of 39°C or more, dehydration, and vomiting with blood due to gastritis. Consult a medical doctor immediately if you or your child experience symptoms of rotavirus infection, as mentioned above. Early examination and treatment may prevent complications of rotavirus infection.

If you or your child is diagnosed with a rotavirus infection, follow the recommendations and therapy indicated by your medical doctor. This is because rotavirus infection can occur more than once, even in people who have received the routine vaccine. To diagnose rotavirus infection, the doctor will ask a question and answer about the symptoms presented by the patient. After that, the doctor will do a physical examination to check for fever and signs of dehydration. In addition, to confirm the diagnosis, the doctor will perform complementary tests, such as: Blood tests, to detect infection and meas-

ure electrolyte levels in the blood to diagnose complications of dehydration. Stool examination to determine the type of germs (microbar) that cause diarrhea and detect rotavirus antigens in the stool sample. Treatment of rotavirus infection. So far there are no medicines that can treat rotavirus infection. However, there are steps that can be taken to relieve symptoms and prevent complications of viruses in the human body. If symptoms are not severe and the patient can still eat or drink, treatment can be done independently at home. These efforts include: Drink lots of water for adults and continue to give milk or formula to babies. Drink orally according to the advice of medical doctor and health professionals. Consume balanced nutritious meals in the form of soups and foods with broth to increase fluid intake. Avoid very sweet or greasy foods. Rest well or enough. If diarrhea gets worse to the point of being difficult to eat and drink, the medical doctor will advise the patient to be hospitalized.

Complications of rotavirus infection. Untreated rotavirus infection can cause complications in the form of: Severe dehydration due to diarrhea, electrolyte imbalance, kidney and liver disorders, acids, and kidney failure is very complicated life.

Prevention of rotavirus infection. There are several ways you can do to prevent and reduce the spread of rotavirus infection, i.e.: Wash your hands with soap and running water, especially after going to the lavatory or bathroom, before and after eating or changing diapers. Vaccination, mainly the rotavirus vaccine is pentavalent, according to the

scheme indicated by the medical doctor. Maintaining the cleanliness and hygiene of the environment around the residence is very important to take more attention the people living in good condition cited by (Tilman CB & Gomes L, 2023).

METHODOLOGICAL RESEARCH

From 2020 to 2022, patients born after January 1, 2019 (date of introduction of rotavirus vaccine in the official calendar of immunizations in Timor-Leste) and at least 12 weeks of age, hospitalized due to acute gastroenteritis in National Hospital of Guido Valadares (HNGV) Dili in the pediatric room were included in this study. Laboratory analyses were performed in the Virology Section of the National Laboratory, adopting the enzymatic immune method (ELISA), based on the RIDASCREEN[®] kit (R-BioPharma AG, Darmstadt, Australia), for the research of rotavirus antigens in feces and serum, with the use of monoclonal antibodies for the product of the sixth viral genome, that is, the VP6 protein, following the manufacturer's recommendations, including determination of optical densities with spectrophotometer using 450 nm filter. Regarding the serum samples, it was observed that recommended by Ray et al⁶, Blunt et al⁸, Chita bar et al⁹ and Patel et al¹¹, adopting about 20 stool and blood samples (serum/plasma) with negative result for rotavirus in order to establish the local validation of the method. Blood samples whose 2-3 sampling standard deviation above the mean of that obtained in negative blood samples was defined as positive. Rotavirus antigens were searched in the patient's feces and serum. Demographic data, disease symptoms and vaccine data

were obtained through interviews with parents and/or consultation with the local medical records. The collected data were compared between the groups with and without antigens. The analysis of clinical data was performed by adopting the universal score of Ruscha and Vesikari¹², and the intensity was classified as moderate, severe and very severe, when scores 1-10, ≥ 11 and ≥ 15 were reached, respectively, in the study conducted cited by (Tilman CB & Gomes L, 2023). Biostatic 5.0 was used to perform statistical analyses. The evaluation of the proportions, involving the parameters mentioned and the present of viral antigen in feces and/or serum, comprised "bivariate" analysis, applying the Chi-square (X^2) tests, with a confidence interval corresponding to 95%. The level of significance adopted was expressed by $p \leq 0.05$ (alfa = 5%).

RESULTS AND DISCUSSION

The study included 156 patients hospitalized with acute gastroenteritis in Nacional Hospital of Guido Valadares (HNGV) Dili, from which 132 paired samples of feces and serum were collected. The search for rotavirus in feces (ELISA) was positive in 14% (24/132) of the samples tested. The majority (14%) of the children whose stool sample was positive for the Rotavirus study were less than 2 years old (58/132). The neighboring state of immunization against Rotavirus was available in 86 patients; of these, 64% (51/56) had received at least one dose of the vaccine and 60% received the complete vaccination schedule (two doses). The antigens were found in 17.5% (14/58) of the patients whose stool samples were positive for Rotavirus. The intensity of symptoms presented by the patients, according to the Ruscha and Vesikari¹² score, was classified as very severe in 40% of those who presented antigens and in 25% of those without circulating Rotavirus antigens in the serum; however, there was no statistically significant difference between both groups indicated. When analyzing the symptoms of Gastroenteritis alone, a difference was observed regarding the number of episodes and duration of vomiting, higher in patients with antigens ($p = 0.012$ and $p = 0.002$, respectively) compared to those without antigens and with positivity for rotavirus in feces. There was no difference between the groups regarding the number of episodes and duration of the diarrhea of the study. In both groups analyzed, 20% of the patients had fever ($> 37.5^\circ\text{C}$), but no relationship was found between antigens and the present fever. The mean duration of hospitalization was three days, both in the antigens group and in the group without antigens, and most patients had leukocytes/mm³ count up to 10,000 in both groups (50% and 70%, respectively). It was found that 60% of the research participants had not been previously hospitalized due to gastroenteritis, and the current episode was the first of severe intensity. The time of disease elapsed, between the onset of gastroenteritis symptoms and serum collection, was on average 2.4 days in patients with antigens and 2.65 days in those without antigens, with no significant difference between groups in table one (1).

Table 1 - Analysis of clinical and laboratory parameters in groups with and without antigens caused by rotavirus in Dili, from 2019 to 2022.

Rotavirus antigenes in sérum (ELISA)					
Parameters	Positive		Negatives		P-value
	N	%	N	%	
Leukocytes/mm ³ ≤10,000 > 10,000	12 19	40.7 19.3	13 25	51 19	0,221
Score Ruska and Vesicare ≥11 (severe) ≥ 15 (Very serious)	12 8	20 10	19 15	25.3 14.7	0.120
Axillar temperature < 37.5 ≥ 37.5	10 11	13 27	18 20	30 30	0.372
Number of vomiting episodes/24h ≤ 3 > 3	7 33	17.5 42.5	25 17	40.3 29.7	0.015
Duration of vomiting (days) ≤ 3 > 3	21 19	32.5 37.5	30 12	40.6 19.4	0.002
Number of episodes of diarrhea/ 24h ≤ 3 > 3	22 19	53.6 46.4	37 31	54.4 45.6	0.939
Duration of diarrhea (days) ≤ 3 > 3	30 10	55 25	37 21	59 31	0.414

Analysis of variance, Chi-square test (χ^2); Score 20 points Ruscha and Vesicaria, 2010; Not available for five patients; Not available for seven patients; Not available to a patient.

In the current context, two oral vaccines against RV, 40% and 80% of cases) in the serum of children with rotaries and rotate[®], are commercialized in several acute gastroenteritis, in this study the detection of countries of the world and, given the possibility that rotavirus antigens in serum occurred in 27.5% of antigens is related to the greater clinical severity of patients who also had it in feces, collaborating the diarrhetic disease and the present of extraintestinal hypothesis of the spread of rotavirus to the blood-manifestations, it is necessary to better understand stream in children with acute diarrhea, according to the finding of antigens in vaccinated populations in the research result. To consider that the primary in-children in Timor-Leste. Since 2019, the oral vac- fection caused by rotavirus is usually more severe cine against human Rotavirus (VORH) is available and that successive infections promote protective in the basic immunization schedule in Autonomous immunity, giving a softer character to subsequent Services of Medicines and Health Equipment, IP infections, it would be reasonable to postulate that Timor-Leste, reached 63% of vaccinal coverage in the large proportion of vaccinated children included the Municipality of Dili Capital of the Nation in this analysis may have influenced the frequency (2022). In the present study, 64%of the children re- with which antigens was found. ceived at least one dose of the RV vaccine, 60% re- Vaccination performed in early stages of life (2 to 4 ceived two doses, and there was no statistically sig- months of age) would be responsible for previous nificant difference in vaccination status between children with and without antigens. Although most and early exposure to rotavirus, which could be a studies demonstrated higher antigens rates (between protective factor against antigens in the present

study, since most of the children evaluated had no

history of previous gastroenteritis. This hypothesis

is supported by findings that demonstrated a negative correlation between antigens and titers of specific antibodies of acute phase against rotavirus in the blood laboratory test official document cited by (Tilman CB & Gomes L, 2023).

Among other possible determinants of protection, it is believed that the antibodies present in the serum, of maternal origin or produced from a previous exposure, would act eventually complicating a viral

hepatogenic spread and limiting infection to the intestine. It is questioned, however, whether infection of antibodies to Rotavirus antigens in the blood would actually be able to prevent antigens or would only influence the ability to detect antigen by ELISA. Studies have described a higher occurrence of antigens among children under 2 years of age, certifying the findings of this research, whose higher frequency of antigens was harmonically higher among lactating women. Additional analyses in vaccinated inhabitants are necessary and probably allow a better understanding of the role played by serum antibodies against antigens caused by rotavirus and acute gastroenteritis. The consequences in this study did not detect an association between rotavirus antigens and clinical severity according to the Ruscha and Vesicaria scores; however, other observations related antigens to the most severe disease when observing significantly higher severity scores among children with rotavirus antigens in the blood, but not vaccinated against this viral cause. At the time of

this study, no statistically significant association was demonstrated between antigens and the higher occurrence of fever, with compatibility with the conse-

quences of other studies.

However, the significant collectivity between antigens and the greater number of episodes and the duration of vomiting, therefore, indicates greater clinical severity, since the present of repeated vomiting hinders oral rehydration therapy and continued feeding and is related to the higher occurrence of electrolyte disorders (particularly hyponatremia), discharging the greater need for intravenous rehydration, intensive care and continued hospitalization.

However, regarding leukocyte scholastic counts, the results of analyses indicate that antigens does not correlate with possible increment of leukocytosis ($> 10,000/\text{mm}^3$), a fact that was also observed studied by (Yun et al, 2018; cited by Tilman CB & Gomes L, 2023). No manifestation and extraintestinal manifestation were observed, although all children included who presented gastroenteritis classified as severe or very severe, suggesting that the possible compromise of other organs and systems would not be accentuated in the presence of antigens associated with rotavirus infection and acute gastroenteritis. Recomposited, in this analysis, there was no significant difference in relation to the time of hospitalization, when compared to children with and without antigens, a fact that is opposed to the findings of Yun et al (2018), who demonstrated significantly longer hospitalization time among children with antigens. In addition, all the children evaluated presented improvement of signs and symptoms at the time of hospital discharge.

The present investigation is a pioneer in the country, when evaluating the antigens caused by rotavirus in

the scenario after the introduction into the basic immunization calendar; however, studies, covering molecular analyses in the samples obtained, are necessary for a better understanding of the pathogenesis of rotaviruses, especially in vaccinated children. In this context, we highlight the need to evaluate whether rotavirus genotypes concomitantly found in fecal specimens and several reserve equivalent or distinct specificities.

Table 2- The coverage of vaccination Pentavalent 1-5 years among children age < 1 year per municipality of Timor-Leste period January-December 2021.

Municipality	Pentavalent I						Pentavalent II						Pentavalent III					
	N			(%)			N			(%)			N			(%)		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
Aileu	860	786	1646	53	49	102	799	818	1617	50	51	100	847	850	1697	53	53	105
Ainoi	898	897	1795	65	64	129	843	859	1702	61	62	122	802	842	1644	58	60	118
Baucau	1981	1870	3851	75	71	146	1824	1773	3597	69	67	137	1850	1817	3667	70	69	139
Bobonaro	1338	1299	2637	68	66	134	1173	1185	2358	59	60	120	1147	1181	2328	58	60	118
Covalima	1095	1056	2151	61	59	119	1036	1059	2095	57	59	116	1049	1075	2124	58	60	118
Dili	5323	5320	10643	54	54	107	5155	5020	10175	52	50	102	5252	5238	10490	53	53	105
Ermera	2375	2364	4739	66	65	131	2171	2127	4298	60	59	119	2195	2252	4447	61	62	123
Lautem	882	849	1731	61	58	119	841	825	1666	58	57	114	833	806	1639	57	55	113
Liquiçá	1285	1172	2457	60	55	115	1272	1237	2509	59	58	117	1353	1193	2546	63	56	119
Manatuto	722	654	1376	71	65	136	677	655	1332	67	65	131	663	608	1271	65	60	125
Manufahi	696	673	1369	56	54	109	693	647	1340	55	52	107	676	630	1306	54	50	104
Oecússi	881	889	1770	75	75	150	848	808	1656	72	69	141	799	756	1555	68	64	132
Viqueque	1148	1039	2187	61	55	115	1090	1009	2099	58	53	111	1127	1006	2133	60	53	113
East Timor	19484	18868	38352	61	59	120	18422	18022	36444	58	56	114	18593	18254	36847	58	57	115

We analyzed the results in (table 3), indicates the vaccination 1, 2, and 3 almost everything in the right vaccination coverage of pentavalent I, II and III line and statistically proven in the result achieved. showed there is a significant difference between all There is national pentavalent level I male 61% and municipalities in the highest vaccination coverage is female 59%, pentavalent II male 58% and female Baucau Municipality and low municipality Aileu. 56% pentavalent III male 58% and female 57% on Baucau pentavalent I male 75% and female 71%, the basis of the research result cited by (Tilman CB pentavalent II male 69% and female 67% pentava-

lent III male 70% and female 69% according to the result of research. The Municipality of Aileu in the

CONCLUSION

category of low vaccination coverage, pentavalent I Rotavirus antigens is a frequent discovered virus male 53% and female 49%, pentavalent II male 50% among children hospitalized with acute gastroenteritis and female 49% of pentavalent III male and female tis caused by this viral agent in Dili Timor-Leste. equal 53% in the result of research. In comparison Among patients with antigens, a higher number of of all municipalities the coverage of pentavalent episodes and duration of signs of vomiting were ob-

served. Rotavirus is the same virus that causes fecal-oral diarrhea is a form of transmission through hands contaminated with feces, accidentally entering the mouth. Signs of rotavirus infection usually appear 2 days after exposure to this virus. The first signs of rotavirus infection are: diarrhea, fever, vomiting and abdominal pain. Symptoms that can appear when dry mouth dehydration occurs, easy to fall asleep, fingertips become cold and consciousness decreased. Meanwhile, signs of rotavirus infection in adults are usually mild. Actually, some patients have no complaints. If it appears, the symptoms of rotavirus contagious that indicate: diarrhea, fever with a temperature of 39°C or more, dehydration and vomiting with blood. However, there are measures that can be taken to relieve symptoms and prevent complications. If the medical doctors were not serious and the patient can still give drink and eat. Treatment can be done independently at home. I recommend that in the future there is more research in this area of knowledge of relevance and continuation of rotavirus and acute gastroenteritis is critical, and the Government should consider this area of research in strengthening vaccination for future generations of Timor-Leste is very important and long-time investments cited by (Tilman CB & Gomes L, 2023).

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