



International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

<http://gssrr.org/index.php?journal=JournalOfBasicAndApplied>



Hospital-Based Pre-Vaccination Surveillance of Rotavirus Gastroenteritis Disease in Infants Less Than 5 Years of Age in the Gambia: 2011-2014

Mr. Sheriffo Jagne*

National Public Health Laboratories, The Gambia

Email: sjagnefa@yahoo.com

Abstract

National Rotavirus Surveillance in the Gambia was initiated in October 2011 to investigate the prevalence and the burden of rotavirus gastroenteritis in infants' < 5 years of age. The aim was to generate data for decision making on the introduction of rotavirus vaccine and to provide base-line data for monitoring the impact of vaccination on the burden of rotavirus disease. Vaccination is the most effective method for preventing rotavirus infection. Pre-vaccination data, on the prevalence and burden of rotavirus genotypes, is crucial in monitoring vaccine efficacy. The Gambia introduced rotavirus vaccine (rota Teq) into routine childhood immunization in August 2013. Sentinel surveillance site at the Gambia's main referral hospital (EFSTH) used the WHO standard Case investigation protocol from 2011-2014. Children under 5 years of age, hospitalized mainly for the treatment of acute gastroenteritis, were enrolled and stool samples were collected and screened for group A rotavirus using Enzyme immunoassay. Confirmed positive cases were genotyped by Polymerase chain reaction at the regional Reference lab in Ghana.

The results shows that from 265 stool samples from enrolled infants (2012-2014), 63 (24%) were ELISA positive for rotavirus. Infants' 0-11 months of age comprised 11% and infants 12-23 months: 8% and 24-59 months: 5%. Rotavirus was detected throughout the year with a peak prevalence period from January to April.

* Corresponding author.

E-mail address: sjagnefa@yahoo.com.

There was mixed rotavirus infection: predominantly genotype G9P [8] in 2013, G12P [8] in 2014. The change in serotypes from 2013 to 2014 was statistically significant. There was an unusual genotype G6P [6] observed which may be due to the result of Zoonotic infection. These results implicated rotavirus infection as a major cause of severe diarrhoea in the Gambia. In the conclusions; we can say that the rotavirus infection is common among Gambian infants. The introduction of the vaccine (rota Teq) in 2013 will serve as a safe and effective intervention to reduce the severity of rotavirus infection. The established surveillance system will continue to monitor and assess the impact and severity of rotavirus disease.

Keywords: Rotavirus; Genotyping; strains

1. Introduction

Rotavirus infection is the highest cause of acute gastroenteritis in children < 5 years of age worldwide [1-2] and is more severe than diarrhoea caused any other enteric pathogens [1,2]. Globally, Rotavirus infection causes about 527,000 deaths per year. The highest proportion of this mortality occurs in low-income countries. Of all deaths caused by rotavirus worldwide, almost half (230,000) are estimated to occur in Africa [3,4]. However, there is only fragmentary data on the rotavirus disease burden from Sub-Saharan Africa for authentication of these estimates [5].

Group A rotaviruses account for the vast majority of human diseases and are classified into different P and G-types based on two outer capsid proteins, VP4 and VP7, Studies have shown that geographical variation in the P and G-type prevalence in different continents, is responsible for the variations in the dominant strains and the emergence of unusual strains globally [6,7]. In both antigenic and genetic variations, 27 G types and 35 P types have been identified among rotavirus strains of both human and animal origins.

Bacteria and other parasitic causes of gastroenteritis are well studied in the Gambia, but there is inadequate data about viral etiologies of diarrhoea. Hospital based rotavirus surveillance in Edward Francis Small teaching Hospital (EFSTH) from 2011 to 2014 showed that rotavirus is the major cause of non-bacterial acute gastroenteritis in children and infants (24%). The Gambia joined the African rotavirus network in 2011 to conduct hospital based surveillance, to estimate the burden of rotavirus gastroenteritis in children less than 5 years of age in the main referral hospital of the Gambia, and to provide evidence based data. The evidence based data generated from the surveillance will assist policy makers to evaluate the need for the introduction of the rotavirus vaccine and to monitor the impact of the vaccine. The main objective of this paper is to describe strain diversity and the epidemiology of rotavirus in the Gambia before the introduction of the vaccine.

2. Methods

The study was conducted from October 2011 through July 2014 in Edward Francis Small Teaching Hospital, formerly known as RVTH. Inclusion and exclusion criteria for diarrhoea cases were as specified in the Regional Office for Africa standard operating procedures and WHO Generic protocol [8].

2.1 Patients

Children less than 5 years of age who were presented with acute gastroenteritis including watery diarrhoea, vomiting, abdominal pain, hospitalized, and with disease symptoms, were enrolled into the surveillance. Gastroenteritis was defined as the occurrence of 3 or more looser stool than normal with or without vomiting. Infants with bloody stool and children above 5 years of age are excluded in the surveillance.

2.2 Stool sample collection

From every enrolled child, approximately 6 ml of stool was collected in screw cap stool collection containers within 48 hours of hospitalization. Stool samples were transported from the surveillance site to the reference laboratory (NPHL) and stored at -20 °C until testing.

2.3 Laboratory diagnosis

Diagnosis of rotavirus infection was determined by ELISA using the rotavirus Prospect test kit. Positive samples and 10% of the negative samples were further characterized at the rotavirus Regional Reference Laboratory in Noguchi Medical research centre, Ghana. The samples were analysed by Polyacrylamide gel electrophoresis (PAGE) to determine the migration of the RNA (long or Short RNA Bands). To determine the rotavirus circulating strains and the samples were also subjected to the Polymerase Chain Reaction (PCR) processes to identify the G and P rotavirus circulating strains in the Gambia.

2. Methods

The study was conducted from October 2011 through July 2014 in Edward Francis Small Teaching Hospital, formerly known as RVTH. Inclusion and exclusion criteria for diarrhoea cases were as specified in the Regional Office for Africa standard operating procedures and WHO Generic protocol [8].

2.1 Patients

Children less than 5 years of age who were presented with acute gastroenteritis including watery diarrhoea, vomiting, abdominal pain, hospitalized, and with disease symptoms, were enrolled into the surveillance. Gastroenteritis was defined as the occurrence of 3 or more looser stool than normal with or without vomiting. Infants with bloody stool and children above 5 years of age are excluded in the surveillance.

2.2 Stool sample collection

From every enrolled child, approximately 6 ml of stool was collected in screw cap stool collection containers within 48 hours of hospitalization. Stool samples were transported from the surveillance site to the reference laboratory (NPHL) and stored at -20 °C until testing.

2.3 Laboratory diagnosis

Diagnosis of rotavirus infection was determined by ELISA using the rotavirus Prospect test kit. Positive samples and 10% of the negative samples were further characterized at the rotavirus Regional Reference Laboratory in Noguchi Medical research centre, Ghana. The samples were analysed by Polyacrylamide gel electrophoresis (PAGE) to determine the migration of the RNA (long or Short RNA Bands). To determine the rotavirus circulating strains and the samples were also subjected to the Polymerase Chain Reaction (PCR) processes to identify the G and P rotavirus circulating strains in the Gambia.

2.4 Data dissemination

Data generated from the surveillance were regularly shared with the Ministry of Health and Social Welfare of the Gambia and WHO both the country office and the regional office.

2.5 Data analysis

Data generated were analysed using the EPI info software. This was to determine the prevalence of rotavirus infection among infants less than 5 years of age in the Gambia, who were hospitalized for acute gastroenteritis. Recording of age distribution and the characterization of the circulating strains were also performed using the same software.

3. Results

In the rotavirus sentinel site in the Gambia, Edward Francis Small Teaching Hospital, a total of 265 stool samples were collected from children less than 5 years of age who were hospitalized with acute gastroenteritis from 2012 to 2014. A total of 63 samples were rotavirus positive for the surveillance period of 3 years.

Table 1: Number of acute diarrhoea cases by year of hospitalization and rotavirus positive cases during the surveillance period from 2012 -2014.

YEAR OF SURVEILLANCE	TOTAL	NEGATIVES	%	POSITIVES	%
2012	19	16	84	3	16
2013	158	124	78	34	22
2014	88	62	70	26	30
TOTAL	265	202	76	63	24

Rotavirus infection was detected in 63/265(24%) of infants that presented with acute gastroenteritis and were hospitalized in ESFTH. Age group distribution from analysis of rotavirus infection: 63 infants were rotavirus positive with the highest rate of the infection manifested in infants within the age bracket of 0-11 mths' (11%), 12-23 months (8%) and 24-56 months (5%).

Rotavirus infection is seasonal in the Gambia and it varies across the surveillance period, reflecting the difference in the climatic conditions. Although rotavirus infection occurs throughout the year, the prevalence of the infection peaked from January to April.

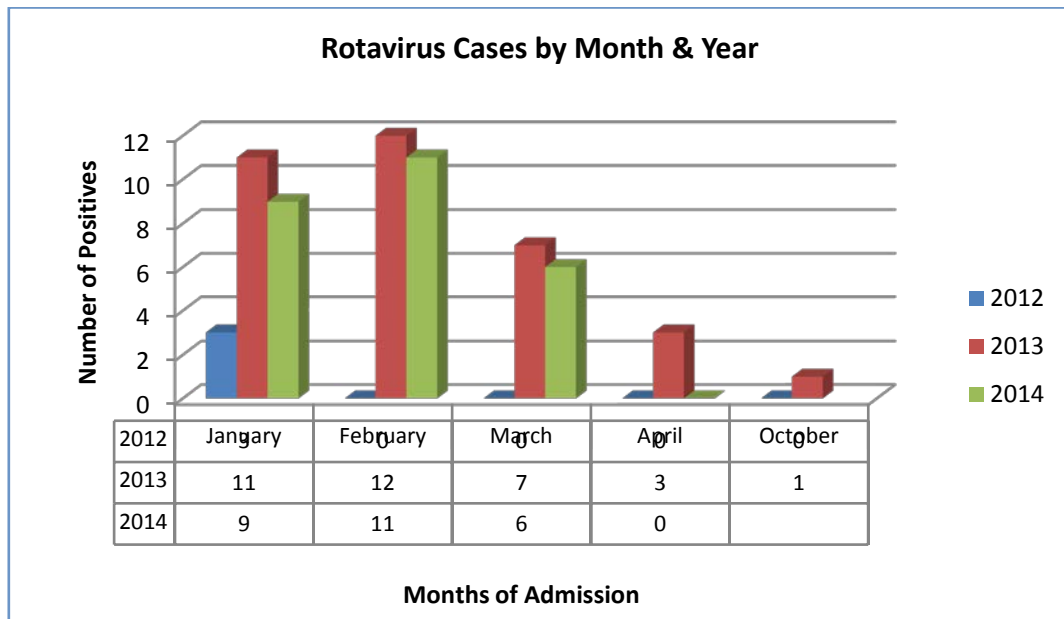


Fig 1: Seasonal Distribution of rotavirus during the surveillance period October- July 2014

Table 2: Distribution of rotavirus G and P Genotypes Circulating in The Gambia 2012-2014

Genotypes	2012 %	2013 %	2014 %	Totals%
G12 P[8]	0 (0%)	6 (23%)	7 (27%)	13(24%)
G1 P[6]	0 (0%)	0 (0%)	6 (23%)	6 (11%)
G1 P[8]	0 (0%)	0 (0%)	3 (12%)	3 (5%)
G1 P[4]	0 (0%)	0 (0%)	1 (4%)	1 (2%)
G- untypeable	0 (0%)	2 (7%)	5 (21%)	7 (13%)
G6 P[6]	0 (0%)	1 (4%)	0 (0%)	1 (2%)
G6 P[8]	0 (0%)	4 (15%)	0 (0%)	4 (7%)
G9 P[8]	0 (0%)	13 (50%)	0 (0%)	13 (24%)
P -untypeable	1 (33%)	0 (0%)	1 (4%)	2 (4%)
G3 P[8]	1 (33%)	0 (0%)	0 (0%)	1 (2%)
G1 PNT	0 (0%)	0 (0%)	0 (0%)	0 (0%)
G-Mixed	1 (33%)	3 (10%)	0 (0%)	4 (7%)
P-Mixed	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Totals	3 (5%)	29 (53%)	23 (42%)	55(100%)

Note: Percentages rounded to the nearest whole number and the total percentage per year is calculated using the total samples genotyped.

Between October 2012 and July 2014, 55 rotavirus positive samples were characterized for G and P types. The most prevalent genotypes detected during this period were G12P [8] (24%) and G9P [8] (24%) followed by G1P [6] (11%), G1P [8] (5%), G6P [8] (7%). Rotavirus genotypes G6P [6], GNTP [6], GNTP [8], G1PNT, G12P [6], G2P [8], G3P [8], G1P [4], G12P [4] were detected with a lower prevalence. There are four samples with mixed genotypes and 9 samples were partially or un-typable for G and P Genotypes. The distribution of the G and P genotypes circulating in the Gambia are shown in the table below. The majority of the circulating strains in the Gambia during the period 2012 -2014 were G12P [8] and G9P [8].

Rotavirus positive cases by age group analysis revealed differences in the infection rate with increasing age. Rotavirus infection within the age bracket of 0-11 months has the highest rotavirus positive rate among the age brackets of infants less than 5 years of age. The positivity rate within this age bracket accounted for 44% of all the positive cases and has contributed to the high burden of the disease within the age bracket of infants 0-11 months. In the other age brackets 12-23 months accounted for 33% of the positive cases and 24-59 months 22% of the positive cases.

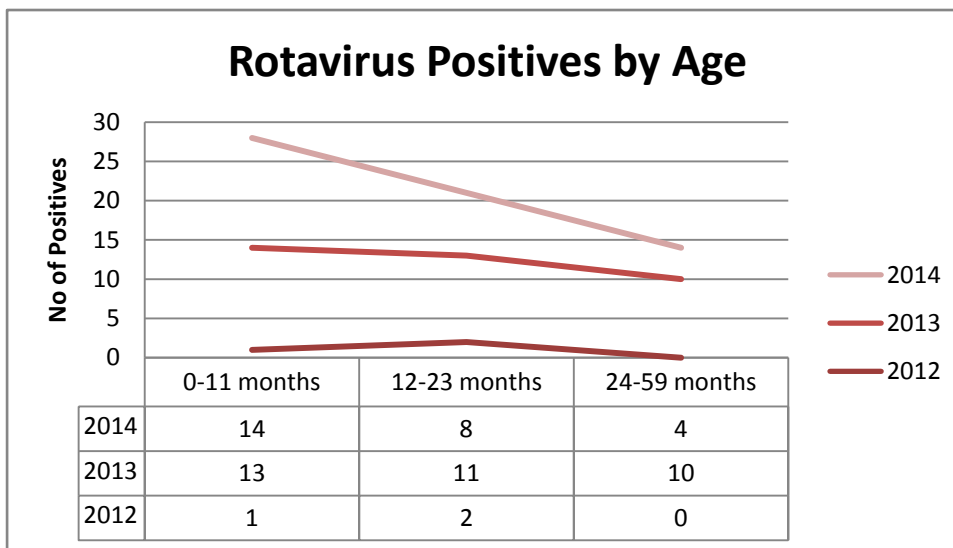


Fig 2. Rotavirus positive cases by age groups 2012 - 2014

5. Discussion

Rotavirus is not a notifiable disease in the Gambia. However, this surveillance estimates the burden of rotavirus infection in the Gambia, based on laboratory data reported through the national rotavirus surveillance. The data produced represent only acute diarrhoea cases, based on WHO case definition criteria that were attributed to rotavirus by the sentinel surveillance site (ESFTH).

The Gambia joined the African Rotavirus Surveillance Network in 2011. From 2011 to July 2014, a total of 265 samples have been collected from infants below 5 years of age who were hospitalized due to acute diarrheal illness. This is similar to the previous study conducted in the URR region in the Gambia over 3 years (2008-2010), which showed the prevalence of rotavirus infection in infants below 5 years of age at 10.6% in 2008, 7.1% in 2009, and 6.5% in 2010 [20]. In a similar study conducted by the Medical Research Council in the Gambia from 2008 -2010 the detection rate of rotavirus infection among children was 20% [26]. In this study, 24% of children who presented with acute gastroenteritis were infected with rotavirus during the surveillance period. During this period before the introduction of the rotavirus vaccine (Rota Teq) 63 samples were positive for rotavirus with the highest prevalence of infection in infants within the age bracket of 0-11 year (11%). Similar to other rotavirus surveillances conducted elsewhere [21, 22], our surveillance confirms the high prevalence of rotavirus infection in infants less than 2 years of age. Therefore, there is a need for the long term protection that can only be achieved through vaccination. Our study observed that rotavirus infection is significantly associated with age and clinical symptoms differ among age groups with vomiting and diarrhoea occurring more frequently (4-5 times a day) among younger patients.

. It was observed that, rotavirus infections occurred throughout the year, with a peak period between January and April in the dry season, with a lot of dust blowing around on the dry winds [23,24]. The seasonality of rotavirus infection in The Gambia is similar to that observed in other countries in West Africa.

During the study period the most prevalent rotavirus genotypes observed were G12P [8] and G9P [8], each with 24% of the total samples collected. This is in line with the prevalence rate in some other African countries according to the Africa rotavirus surveillance report 2006-2008. The incidences recorded: in Uganda G9P [8] (16%), Cameroon G9P [8] (19%) and G12P [8] (10%) and Ethiopia G12P [8] (8%). These genotype results are different from the genotypes obtained by MRC during their rotavirus study in one of the regions in rural Gambia (URR) from 2008-2010. The predominant genotypes from their study were G2P [6] 28%, G1P [8] 26% and G1P [10] 10% and the rare identified genotypes were 1% [26]. There was one unusual genotype observed during the surveillance period, G6P [6], which might be due to potential reassortment between human and animal rotavirus strains due to zoonotic infection. Mixed infection was observed in the G-types of two samples during the study period (G1G12P [8] and G12G1P [8]). The genotype results also show a number of untypeable rotavirus strains (15%). It is possible that these are unusual rotavirus strains of animal origin. Alternatively they could be due to the result of primers used for typing by reverse transcriptase polymerase chain reaction that do not represent consensus sequences in these animal derived rotavirus strains [7].

The majority of the positive samples processed on Poly Acrylamide Gel Electrophoresis (PAGE) were predominantly long RNA (77%) and few were found to have short electrophoretic pattern (23%).

The Gambia introduced the rotavirus vaccine (Rota Teq) in 2013, but we have not started the rotavirus vaccine impact study and the intussusceptions study. As a result, the information on vaccine received by infants during the surveillance period was not collected. Due to these factors the potential effects of vaccination on the incidence of acute rotavirus gastroenteritis and the prevailing rotavirus types could not be determined.

There are some limitations in the surveillance activities especially in the recruitment of cases and sample collection. One of the limitations of this surveillance is the potential error in the estimation of the rotavirus incidence rate, as the surveillance was conducted in only one site, which does not include other hospitals that admit paediatric acute gastroenteritis cases. The only surveillance site was located in the only major referral hospital in the capital city Banjul (ESFTH). As a result the data generated may not be a true representation of the prevalence and burden of rotavirus in the Gambia. Therefore there is a need to increase the surveillance sites to other areas in the greater Banjul as well as in the rural areas for better understanding of the true burden of rotavirus infection in the Gambia. The rotavirus-positive samples that were collected and genotyped may not represent the circulating rotavirus strains throughout the Gambia. However, the predominant strains were captured and their diversity has been demonstrated.

6. Conclusion

These data highlight the prevalence of Rotavirus in infants who were hospitalized with acute gastroenteritis and Diarrhoea which has been demonstrated in the extreme ends of the country [26]. Rotavirus is common in Gambian infants, hence the need for safe and effective interventions. The predominant circulating strains have changed from 2013 to 2014, G12P [8] and G9P [8] respectively. It is important to study the diversity of rotavirus strains and to monitor the impact of rotavirus vaccination on the severity of rotavirus infection in the Gambia.

Acknowledgements

The authors would like to thank the infants and their families for participating in the surveillance, The Gambia Ministry of Health and Social Welfare, Doctors and the Nurses working at the surveillance site, World Health Organization Country Office, EPI and all the staff from the National Public Health Laboratories for their contribution and support.

References

- [1] Albano F, Bruzzese E, Bella A, et al. *Rotavirus and not age determines gastroenteritis severity in children: a hospital based study*. Eur J pediatr.;166:241-247: 2007.
- [2] Kapikian AZ, Channock RM. Rotavirus. In: Fields BN, Knipe DE, Howley PM (eds). *Fields Virology*. Philadelphia: Lippincott-Rave Publisher;:1657-1707: 1996.
- [3] Pashar UD, Gibson CJ, Bresee JS, *Glass RI. Rotavirus and severe child hood diarrhea*. Emerg infect Dis; 12:304-306. 2006
- [4] World Health Organisation. *Estimated rotavirus deaths for children under 5 years of age, 2004* http://www.who.int/immunization_monitoring/burden/rotavirus_estimates/en/. Accessed 27 April 2009.
- [5] Molbak K, Fisher TK, Mikkelsen CS. The estimation of mortality due to rotavirus infections in Sub-Saharan Africa. *Vaccine* 19:393-395; 2000

- [6] Estes MK, Kapikian AZ. Chapter 53: rotaviruses. In: Knipe DM, Howley PM, Griffin DE, et al., eds. *Fields virology*. 5th ed. 2. Philadelphia: Lippincott, William & Wilkins 1917-1958; 2007;
- [7] Mwenda JM, Ntoto KM, Abebe A, et al. Burden and epidemiology of rotavirus diarrhea in selected African countries: Preliminary results from the African rotavirus Surveillance network. *J infect Dis.*; 202:S5-S11; 2010
- [8] Abebe, Almaz, Telahun Teka, Tassew Kassa, Mapaseka Seheri, Berhane Beyene, Birke Teshome, Ferehiwot Kebede, et al. 2014. "Hospital-Based Surveillance for Rotavirus Gastroenteritis in Children Younger than 5 Years of Age in Ethiopia: 2007-2012." *The Pediatric Infectious Disease Journal* 33 Suppl 1 (1): S28–33. doi:10.1097/INF.0000000000000048.
- [9] Akoua-Koffi, Chantal, Vincent Asse Kouadio, and Jean Jacques Yao Atteby. 2014. "Hospital-Based Surveillance of Rotavirus Gastroenteritis among Children under 5 Years of Age in the Republic of Ivory Coast: A Cross-Sectional Study." *BMJ Open* 4 (1): e003269. doi:10.1136/bmjopen—003269; 2013
- [10] Chang, Wan-Chi, Catherine Yen, Fang-Tzy Wu, Yhu-Chering Huang, Jen-Shiou Lin, Fu-Chen Huang, Hui-Tzu Yu, et al.. "Effectiveness of 2 Rotavirus Vaccines against Rotavirus Disease in Taiwanese Infants." *The Pediatric Infectious Disease Journal* 33 (3): e81–6. doi:10.1097/INF.000000000000105. 2014
- [11] Document, Technical. "Impact of Rotavirus Vaccination – Generic Study Protocol."
- [12] Ichihara, Maria Y T, Laura C Rodrigues, Carlos a S Teles Santos, Maria Da Gloria L C Teixeira, Sandra R De Jesus, Sheila M Alvim De Matos, Jose P Gagliardi Leite, and Mauricio L Barreto. 2014. "Effectiveness of Rotavirus Vaccine against Hospitalized Rotavirus Diarrhea: A Case-Control Study." *Vaccine* 32 (23). Elsevier Ltd: 2740–47. doi:10.1016/j.vaccine..01.007. 2014
- [13] Martínón-Torres, Federico, Angela Aramburo, Nazareth Martínón-Torres, Miriam Cebey, María Teresa Seoane-Pillado, Lorenzo Redondo-Collazo, and Jose Maria Martínón-Sánchez.. "A Reverse Evidence of Rotavirus Vaccines Impact." *Human Vaccines & Immunotherapeutics* 9 (6): 1289–91. doi:10.4161/hv.24182. 2013
- [14] Mullick, Satarupa, Paulami Mandal, Mukti Kant Nayak, Souvik Ghosh, Papiya De, K Rajendran, Mihir K Bhattacharya, et al. 2014. "Hospital Based Surveillance and Genetic Characterization of Rotavirus Strains in Children (<5 Years) with Acute Gastroenteritis in Kolkata, India, Revealed Resurgence of G9 and G2 Genotypes during 2011-2013." *Vaccine* 32 Suppl 1 (August). Elsevier Ltd: A20–8. doi:10.1016/j.vaccine..03.018. 2014
- [15] Oishi, Tomohiro, Tetsuo Taguchi, Tokushi Nakano, Shoji Sudo, and Hiroaki Kuwajima. "The Occurrence of Severe Rotavirus Gastroenteritis in Children under 3 Years of Age before and after the Introduction of Rotavirus Vaccine: A Prospective Observational Study in Three Pediatric Clinics in Shibata City, Niigata Prefecture, Japan." *Japanese Journal of Infectious Diseases* 67 (4): 304–6. <http://www.ncbi.nlm.nih.gov/pubmed/25056079>. 2014
- [16] Pendleton, Annmarie, Maja Galic, Christopher Clarke, Su Peing Ng, Emilio Ledesma, Gunasekaran Ramakrishnan, and Yanfang Liu. "Impact of Rotavirus Vaccination in Australian Children below 5 Years of Age: A Database Study." *Human Vaccines & Immunotherapeutics* 9 (8): 1617–25. doi:10.4161/hv.24831. 2013.

- [17] Phua, Kong Boo, Nancy Tee, Nancy Tan, Gunasekaran Ramakrishnan, Yee-Leong Teoh, Hans Bock, and Yanfang Liu.. "A Hospital-Based Surveillance of Rotavirus Gastroenteritis in Children <5 Years of Age in Singapore." *The Pediatric Infectious Disease Journal* 32 (12): e426–31. doi:10.1097/INF.0b013e31829f2cb0; 2013
- [18] Saluja, T, S D Sharma, M Gupta, R Kundu, S Kar, a Dutta, M Silveira, et al. 2014a. "A Multicenter Prospective Hospital-Based Surveillance to Estimate the Burden of Rotavirus Gastroenteritis in Children Less than Five Years of Age in India." *Vaccine* 32 Suppl 1 (August). Elsevier Ltd: A13–9. doi:10.1016/j.vaccine.03.030. 2014
- [19] Vesikari, Timo, Matti Uhari, Marjo Renko, Maria Hemming, Marjo Salminen, Laurence Torcel-Pagnon, Hélène Bricout, and François Simondon.. "Impact and Effectiveness of RotaTeq® Vaccine Based on 3 Years of Surveillance Following Introduction of a Rotavirus Immunization Program in Finland." *The Pediatric Infectious Disease Journal* 32 (12): 1365–73; 2013
- [20] The Gambia, Rotavirus disease population-based surveillance in the Upper River Region of the Gambia (unpublished 2010)
- [21] Bizuneh T, Mariam ZS, Abebe A, Et al. Rotavirus infection under-five children in Jimma Hospital, Southwest Ethopia. *Ethiop J Health Dev.*, 18:19-24, 2004
- [22] Vesikari T, Karvonen A, Prymula R, et al Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomized, double-blind controlled study. *Lancet*;370:1757-1763, 2007
- [23] Tazi-Lakhsassi L. Epidemiological and clinical study and electropherotyping survey of rotavirus acute diarrhea in children's infectious disease unit in Casablanca, Morocco. *Ann Inst Pasteur Virol.*139:205-215; 1988
- [24] Banyai K, Bogda'n A, Domonkos G, et al. Genetic diversity and zoonotic potential of human rotavirus strains, , Hungary. *J Med Virol.*2009;81:362-370.3; 2003-2006
- [25] Mwenda, Jason M, Kinkela Mina Ntoto, Almaz Abebe, Christabel Enweronu-Laryea, Ismail Amina, Jackson Mchomvu, Annet Kisakye, et al. "Burden and Epidemiology of Rotavirus Diarrhea in Selected African Countries: Preliminary Results from the African Rotavirus Surveillance Network." *The Journal of Infectious Diseases* 202 Suppl (Suppl 1): S5–S11. doi:10.1086/653557; 2010.
- [26] Kwambana, Brenda A., Usman N. Ikumapayi, Neneh Sallah, Michel Dione, Sheikh Jarju, Sandra Panchalingham, James Jafali et al. "High Genotypic Diversity among Rotavirus Strains Infecting Gambian Children." *The Pediatric infectious disease journal* 33 S69-S75(2014).