

Rotavirus as a foodborne pathogen: a serious global health concern

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Abstract

Viral food borne infections are extremely hazardous and often overlooked when instating food safety guidelines. Rotavirus is one of the most common and most deadly food borne viral pathogens. It threatens the life of *literally* every single individual born in this world. It is a tri-layered retrovirus that invades the cells of the intestinal epithelia. Rotavirus infects primarily mammals and disease transmission takes place through the fecal-oral route. All children under the age of 5 are at risk of contraction and fatality. The infection is primarily manifested by painful gastroenteritis and profuse diarrhea. Dehydration and septic shock that stems from disease progression is generally the cause of death. The standard diagnostic assay for the pathogen is retro-PCR, and the symptoms are treated with progressive rehydration therapy. The risk of rotavirus contraction is prevalent throughout the globe, although third world countries and particularly the countries of South Asia and Africa endure the heaviest burden of disease. Rotarix and RotaTaq are highly efficacious and globally approved vaccines for rotavirus. In the context of Bangladesh, rotavirus is particularly important because the current rotavirus vaccines are quite expensive for the economy of developing nations, and for unknown reasons, display faltered effectiveness in the population. The development of an indiscriminately effective and affordable rotavirus vaccine for developing nations such as Bangladesh is an avenue for much commercial interest.

Keywords: Food Borne Pathogen; Rotavirus; Gastroenteritis; Diarrhea; Bangladesh

Introduction

A large fraction of foodborne illnesses is caused by viruses. It has been reported that viral infections cause more than 5 million foodborne illnesses each year in USA alone [1]. These intracellular parasites are small, numerous and evasive, allowing them to easily contaminate food materials. Most foodborne viruses target the cells of the intestinal epithelium. Of the harmful foodborne viruses, the most notable are noroviruses, rotaviruses, adenoviruses, astroviruses, enteroviruses, hepatitis A and E viruses, coronaviruses, parvoviruses, adenoviruses and aichi viruses [2]. Rotavirus is responsible for profuse diarrhea and painful gastroenteritis in young children. It has been reported that nearly every child in the world that is under 5 years of age is

at risk of infection by group A rotavirus [3]. The virus causes an estimated 450 thousand deaths, over 2 million hospitalizations and 25 million outpatient cases annually, with over 90% incidence in developing countries of Asia and Africa [4].

Rotavirus is a member of the Reoviridae family of RNA viruses, discovered around 40 years ago in murine intestines [5]. About 10 years later, it was identified as the cause of acute gastroenteritis in children [6]. It has a discrete 11-segmented double-stranded RNA genome. It is a wheel-shaped, 70nm diametric, non-enveloped virus with three concentric protein layers coating the genetic material [7]. The first core shell, VP2 surrounds two proteins VP1 (polymerase), VP3 (capping enzyme molecules) and the genomic RNA [8]. A middle layer of protein VP6 surrounds the core shell and an outer layer made of VP4 and VP7 encloses the complete structure [9]. During infection, the outer layer is lost and a double-layered particle is injected into the host cell as the payload [8]. Entry into the host cell is mediated by various cell surface molecules, including sialic acid [10], heat shock cognate protein 70 [11], integrins [12] and protein disulfide isomerases [13]. Upon host cell invasion, positive-strand mRNAs are produced which are simultaneously used for viral protein synthesis and the synthesis of genomic RNA. Viral protein and RNA synthesis occur in dense structures called viroplasm that appear in the cytoplasm promptly after infection [14]. Assembly of new viral particles are completed in the ER lumen where they acquire the VP4 (protease sensitive protein) and VP7 (glycoprotein) constructed outer layer [15]. Release of complete viral progeny takes place through cellular lysis or a unique vesicular transport pathway [16].

The importance of this study is to shed light on the health impact and disease burden of viruses as foodborne pathogens while focusing on rotavirus.

Classification

Rotaviruses are members of the Reoviridae family based on homology between structure, genome organization and

replication strategy. There are 8 recognized classes (RVA-RVH) and two proposed classes (RVI and RVJ) of Rotavirus [17]. For humans and mammalian hosts RVA is the most common to infect (over 90% cases), followed by RVC, RVE, RVH and RVI [18]. RVAs are further classified into 4 subgroups on the basis of the VP6 protein and 2 geno groups on the basis of sequence data [19].

It is primarily the two outer capsid antigens, VP7 and VP4 that determine the genotype specificity. VP7 is a glycoprotein antigen and VP4 is a protease sensitive antigen. Antigen based detection methods have segregated up to 35 G type (VP7) and 50 P type

(VP4) genotypes [20,21]. Based on the differences in the migration pattern of genome segments 10 and 11 in polyacrylamide gels, there are two major RNA profiles of rotavirus designated as the long and short profiles [22]. Originally, emphasis was on immunological methods to identify viral serotypes. Presently, there is an ongoing nucleotide sequence identity based approach. Multiple types of 11 different classes derived from the 11 protein coding genes on rotavirus continue to be typed [23]. The most common G-genotypes are G1, G2, G3, G4, G9 and G12, and the most common P-types are P4, P6 and P8, collectively assumed to be responsible for almost 90% global incidence of infection [24].

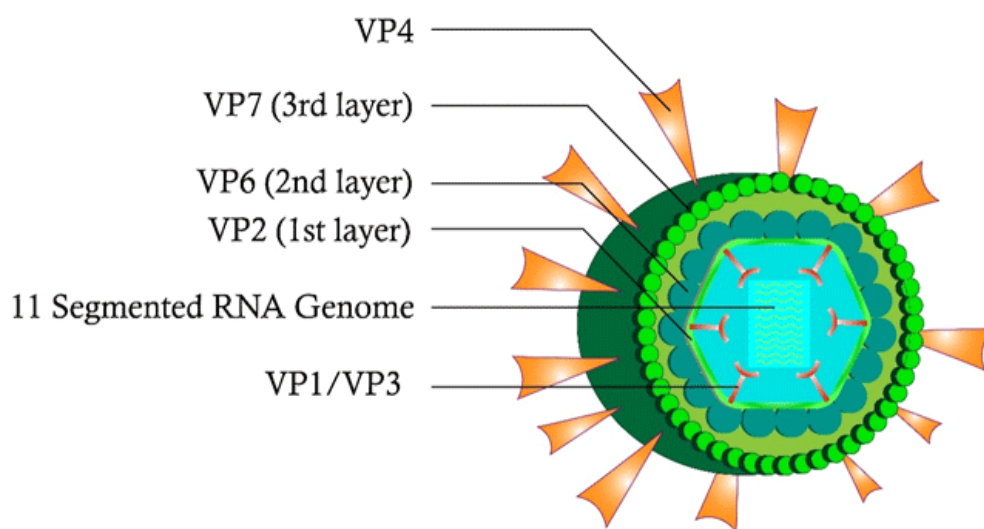


Figure 1: Schematic of Rotavirus Structure. Rotavirus has an 11-segmented double-stranded RNA genome encapsulated within 3 layers of viral proteins.

Pathogenicity and Transmission

Rotavirus infection antagonizes the small intestine, specifically the mature, differentiated enterocytes found at the tips of the villi. The infectious dose is estimated to be between a hundred and a thousand virus particles [25]. In immune compromised individuals, the spread of the infection to extra-intestinal organs has also been documented [26]. Multiple factors determine the pathogenicity and possible outcome of the disease, most predominantly, the age of the patient. Immunity acquired by the transplacental and colostrum-mediated transfer of maternal antibodies protect children under three months of age from symptomatic disease [27]. Maximum susceptibility to disease resides between ages 3months and 24months.

After an incubation period of 1 to 3 days, the illness manifests itself with severe impact. Symptoms include abdominal pain, fever, profuse diarrhea, bulging of the midsection, dehydration, fatigue, dizziness and vomiting. Diarrhea is caused by disruption

of absorptive and secretory functions of the intestinal cells. Death and desquamation of the villous cells and the proliferation of secretory crypt cells induces abdominal discomfort and looseness of the bowels [28]. The illness is exacerbated by the resulting decrease in signaling molecules and digestive enzymes, as well as paracellular leakage from the ransacked tight junctions between the enterocytes [29].

Although common between other gastrointestinal pathogens, the symptoms tend to be abruptly severe in rotavirus infections. This is why rotavirus induced gastroenteritis patient's end up in the hospital much more frequently than similar symptoms of other pathogenic origins [30]. The diarrhea characteristic of the disease has been attributed to several different mechanisms. The virus causes destruction of enterocytes and disruption of the absorptive intestinal epithelia. Other effects that may confer diarrheal symptoms include villus ischemia, enterotoxin activity of the viroid, secretion of calcium and chloride ions with intracellular fluid by NSP4, and the activation of distal nerve

impulses that indirectly increase enterogastric secretions [31, 32].

Epidemiology

Nearly every child in the world under 5 years of age is at risk of infection by group A rotavirus [3]. The burden of disease for this virus is astoundingly high. An estimated 20% to 30% of all cases of acute gastroenteritis at hospitals are attributed to rotavirus infection [30]. One in every 7 premature children deaths in Europe is attributed to rotavirus infection, accounting for a total of 231 deaths, over 87 thousand hospital visits and nearly a million outpatient visits [33]. The mortality rates are highest in South Asian countries such as Bangladesh, India, Pakistan, Nepal, Sri Lanka and African countries such as Nigeria and Congo [4]. A report in 2013 finds half of all rotavirus deaths in the world to take place in India, Nigeria, Pakistan and the Democratic Republic of Congo.

Although sporadic and endemic cases of rotavirus infection occur throughout the year, infection rates are highest in the winter when the air is dry and the atmosphere is cool [34]. Developing countries where people are plagued by poor sanitation and waning health facilities, rotavirus induced diarrhea takes on the most horrific form. Adults are rarely infected due to the immunizing effect of early encounters. Children under 14 months of age are at a higher risk than older children and immune compromised children are at a higher risk than immune competent children [35].

The viral particles are extremely resistant to harsh environmental conditions and dangerously contagious. The fecal-oral route is the primary mode of transmission. Infections spread through contamination of food items, drinking water, utensils and

exposure to contaminated hands and surfaces [36]. In developed countries, the majority of infections are of nosocomial origins [37].

Lifecycle

An infectious rotavirus virion begins its lifecycle through its attachment to specific enterocyte surface molecules [10-13]. The attachment process and viral specificity is quite complex. After attachment, the outermost layer is shed as the double-layered payload enters the host cell. The entry takes place either through receptor mediated endocytosis, clathrin-mediated endocytosis or by other mechanisms [38]. Shortly after entry, viroplasm appears in the cytoplasm formed by yet unknown mechanisms. The viroplasm acts as the vantage point for replication and assembly of nascent virus particles.

The payload is transcriptionally activated once in the cytoplasm. The transcriptional complex is composed of the RNA dependent RNA polymerase VP1 and the viral capping enzyme VP3 complexed with the segmented RNA genome [39]. Positive sense mRNA transcripts are produced for several rounds. Viral proteins and genomic RNA are synthesized simultaneously [40].

Double-layered virus particles stem from the viroplasm and move to the endoplasmic reticulum by the coordinated action of NSP4 and VP6 [41]. Once the outer layer is assembled, virus particles exit the cell either by lysis or by secretory surface release. The exact mechanisms to the exit from the host cell are still under extensive study [42]. It takes about 10 to 12 hours to complete the lifecycle from attachment to progeny formation. In children the infection rapidly transfuses around the host cell releasing newly synthesized virus particles [43].

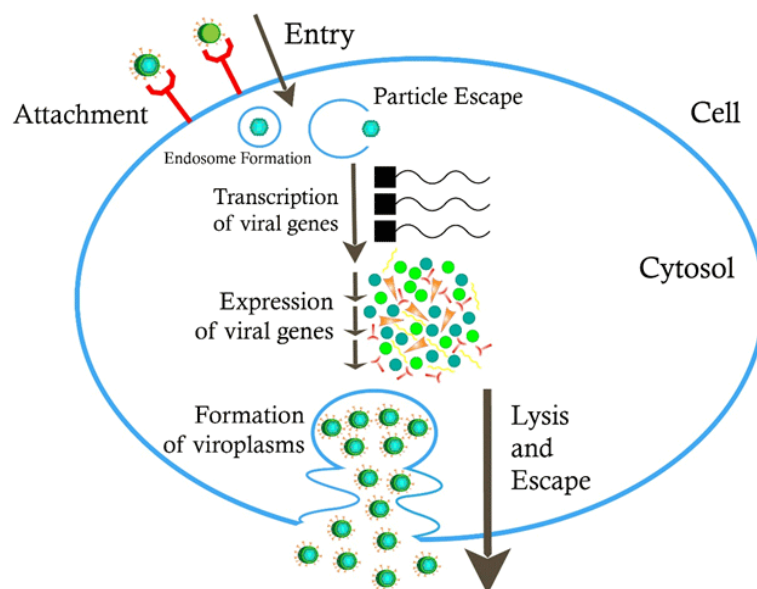


Figure 2: Rotavirus Lifecycle. After attachment and entry, viral genes are expressed in the cytosol and viral progeny are assembled within the newly formed viroplasm, until eventually new virions lyse out of the cell.

Diagnosis

Rotavirus infection is primarily diagnosed by laboratory detection in fecal samples. Electron microscopy, polyacrylamide gel electrophoresis, immunoassays, retro PCR, virus isolation and other advanced detection methods are generally employed [44]. Because of its efficacy in pathogenicity, during the acute phase of the infection, large masses of viral particles can be found in the fecal matter. Following detection, electrophoretic migration aids the narrowing down of specific strains [45]. However, it is not protocol to test children with gastroenteritis directly for rotavirus since diagnosis generally does not alter the treatment scheme. The use of commercially available ELISA kits and immune chromatographic assays have improved the diagnostic procedure with their unprecedented specificity and sharp sensitivity [46]. For diagnostic genotyping, protocols for reverse transcriptase polymerase chain reactions (RT-PCR) are available [47].

Treatment

Treatment of rotavirus infection is focused around dehydration and gastroenteritis alleviation by rejuvenating enteric cells. In most cases noninvasive treatment begins before diagnosis. Hydration levels are monitor and general medication of gastrointestinal discomfort is prescribed. Oral or intravenous rehydration therapy is practiced. For mild to moderate levels of diarrheic dehydration, the oral route is sufficient [48]. For children who are severely dehydrated and terribly showing signs of shock and delirium, intravenous rehydration is recommended.

The WHO suggested glucose based oral rehydration blend is composed of 90mM sodium and 111mM glucose with a total osmolarity of 311mM [49]. For non-cholera based diarrhea, WHO recommends a different standard with lower osmolarity containing 75mM sodium and 75mM glucose with a total osmolarity of 224mM that is found to incite better response with lesser complications [49]. For children with minimal to no signs of dehydration roughly 100mL of rehydration solution is recommended for every time fluid is lost from the bowels. For suckling infants, breastfeeding should continue throughout the rehydration and treatment process even though lactose intake is generally discouraged in the event of diarrhea since there is a lack of evidence for lactose being detrimental to gastroenteritis patients [50]. Zinc supplementation improves the rehydration regime and is recommended by WHO standards.

In addition to rehydration therapy, inclusion of other supplements such as amino acids, lactoferrins, lysozyme and probiotic compounds are under investigation. Probiotics with *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* were found to shorten the duration of the illness [51]. Prebiotic components such as human milk oligosaccharides (HMO), short chain galactooligosaccharides (scGOS), long chain fructooligosaccharides (lCFOS) and pectin derived acidic

oligosaccharides have shown tremendous promise and efficacy in reducing the symptoms of infection [52].

Immunization against Rotavirus

Multiple rotavirus vaccines have so far been successfully employed in the control and prevention of the health crisis [53]. The burden of disease has been greatly assuaged in large proportions of the developing countries in Africa and Asia [54]. Two vaccines, Rotarix (RV1) and RotaTeq (RV5) are proven effective against homotypic, heterotypic and partly heterotypic virulent rotavirus strains [55]. Since 2009, expanded programs on immunization against rotavirus have been in effect all around the world with much needed emphasis on the developing world [56]. For unknown reasons, the efficacy of the vaccines has been found to be 30 to 40% lower in developing countries than developed nations [57].

Rotavirus vaccines have a history of inducing risk of intussusceptions (ISs) [55]. The RV1 Rotarix vaccine is a monovalent live-attenuated vaccine that benignly replicates in the gut until identified and eliminated by the adaptive immune system causing immunization. The vaccine displays high efficacy with minimal risks of intussusceptions [58]. The RV5 RotaTeq is a pentavalent live-attenuated vaccine developed from genetic reassortment of human and bovine strains. Due to its compromised replication efficiency compared to Rotarix, the initial immunization is disparate and requires multiple doses at select intervals [59]. This vaccine also demonstrates high efficacy and insignificant risks [60].

Rotavirus in Bangladesh

Foodborne illnesses have always plagued Bangladesh. Because of the economically compromised state of the majority of the nation, public infrastructure, especially in terms of health, has been substandard. Living standards, sanitation and food hygiene is alarmingly poor. For a pathogen such as rotavirus, the gateway to causing massive etiological damage has been wide open. Among other foodborne pathogens, rotavirus induced gastroenteritis and diarrhea pose serious threats and health risks in the great majority of underdeveloped localities in Bangladesh.

As a developing country, the burden of rotavirus infection in Bangladesh has consistently been high [57]. Both globally marketed vaccines RotaTeq and Rotarix demonstrate lower efficacy in Bangladesh, compared to developed nations [57]. This loss of impact in vaccination is not a economy and sanitation issue but rather attributed to the genotypic differences of common prevailing rotavirus strains in Bangladesh in regard to the origins of the vaccines themselves [61]. The genetic makeup of the individual and influence of maternal antibodies in the body of the child could also play a part here [62, 63]. A hospitalization of children due to diarrheal sickness has prominently been one of

largest concerns in Bangladesh for many decades [64]. Studies in the capital city of Dhaka, Bangladesh, which is arguably the most developed part of the country, show over 1/3rd of the children admitted to hospitals to be positive for rotavirus infection [65]. Rain and flooding in late summer accelerate the rate of disease transmission. Multiple serotypes of the virus have been found to prevail and tactfully cause disease in the population [66].

Each dose of the rotavirus vaccine comes in at over 20USD in Bangladesh which is very high considering the per capita income in Bangladesh has only recently crossed 1,500USD [67]. Because of the intemperate cost, there has been reluctance in launching nationwide mandatory vaccination programs against rotavirus and until and unless a vaccine demonstrating higher efficacy is introduced, it is unlikely that the bill will pass.

Conclusion

Viral contamination of food takes place more frequently and is much more difficult to control compared to larger, live pathogens. Most current hygiene guidelines have been established for prevention of bacterial contamination of food, granting a free passage to pathogenic viruses. This negligence towards viral infections that are transmitted through the oral route pose a greater threat to food safety and *one health*. When we look at the statistics for deaths and hospitalizations by virtue of rotavirus, the numbers appear truly staggering. In any conducted study, rotavirus commands a formidable position as a foodborne pathogen. For a developing country such as Bangladesh, foodborne illnesses that stem from poor sanitation and inferior infrastructure are of the utmost health concern. Contaminated food and drinking water give rise to infallible losses in public health and manpower. Rotavirus is a highly prevalent infectious agent in Bangladesh and around the world, affecting almost every single child that is born in their early lives. All biological evidence suggests rotavirus to be largely a vaccine preventable disease. Development of an indiscriminately effective and affordable rotavirus vaccine for developing nations such as Bangladesh is an avenue for much commercial interest.

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Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been

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References

1. Koopmans M and E Duizer. Foodborne viruses: an emerging problem. *Int J Food Microbiol.* 2004;90(1):23-41.
2. Shukla S, Cho H, Kwon OJ, Chung SH, Kim M. Prevalence and evaluation strategies for viral contamination in food products: Risk to human health-a review. *Crit Rev Food Sci Nutr.* 2018;58(3):405-419. Doi: 10.1080/10408398.2016.1182891
3. World Health Organization. Rotavirus vaccines. *Relev Epidemiol Hebd.* 2007;285-295.
4. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis.* 2012;12(2):136-141. Doi: 10.1016/S1473-3099(11)70253-5
5. Adams WR and L M Kraft. Epizootic diarrhea of infant mice: identification of the etiologic agent. *Science.* 1963;141(3578):359-360.
6. Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. *Lancet.* 1973;2(7841):1281-1283.
7. Yeager M, Dryden KA, Olson NH, Greenberg HB, Baker TS. Three-dimensional structure of rhesus rotavirus by cryoelectron microscopy and image reconstruction. *J Cell Biol.* 1990;110(6):2133-2144.
8. McClain B, Settembre E, Temple BR, Bellamy AR, Harrison SC. X-ray crystal structure of the rotavirus inner capsid particle at 3.8 Å resolution. *J Mol Biol.* 2010;397(2):587-599. Doi: 10.1016/j.jmb.2010.01.055
9. Settembre EC, Chen JZ, Dormitzer PR, Grigorieff N, Harrison SC. Atomic model of an infectious rotavirus particle. *Embo j.* 2011; 30(2):408-416. Doi: 10.1038/emboj.2010.322
10. Thomas Haselhorst, Fiona E Fleming, Jeffrey C Dyason, Regan D Hartnell, Xing Yu, Gavan Holloway, Kim Santegoets, Milton J Kiefel, et al. Sialic acid dependence in rotavirus host cell invasion. *Nat Chem Biol.* 2009;5(2):91-93.
11. Guerrero CA, Bouysounade D, Zárate S, Isa P, López T, Espinosa R, Romero P, Méndez E, López S, et al. Heat shock cognate protein 70 is involved in rotavirus cell entry. *J Virol.* 2002;76(8):4096-4102.
12. Zárate S, Romero P, Espinosa R, Arias CF, López S. VP7 mediates the interaction of rotaviruses with integrin alphavbeta3 through a novel integrin-binding site. *J Virol.* 2004;78(20):10839-10847.
13. Calderon MN, Guerrero CA, Acosta O, Lopez S, Arias CF. Inhibiting rotavirus infection by membrane-impermeant thiol/disulfide exchange blockers and antibodies against protein disulfide isomerase. *Intervirology.* 2012;55(6):451-464. Doi: 10.1159/000335262
14. Petrie BL, Greenberg HB, Graham DY, Estes MK. Ultrastructural localization of rotavirus antigens using colloidal gold. *Virus Res.* 1984;1(2):133-152.
15. Estes M KA. Rotaviruses. *Fields of Virology.* 2007.
16. Tomas López, Minerva Camacho, Margarita Zayas, Rebeca Nájera, Rosana Sánchez, Carlos F. Arias, Susana López, et al. Silencing the

- morphogenesis of rotavirus. *J Virol.* 2005;79(1):184-192. Doi: 10.1128/JVI.79.1.184-192.2005
17. Bányai K, Kemenesi G, Budinski I, Földes F, Zana B, Marton S, Varga-Kugler R, et al. Candidate new rotavirus species in Schreiber's bats, Serbia. *Infect Genet Evol.* 2017;48:19-26. Doi: 10.1016/j.meegid.2016.12.002
18. Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M, John R. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. *Arch Virol.* 2012;157(6):1177-1182. Doi: 10.1007/s00705-012-1273-3
19. Iturriza Gómara M, Wong C, Blome S, Desselberger U, Gray J. Molecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. *J Virol.* 2002;76(13):6596-6601.
20. Li K, Lin XD, Huang KY, Zhang B, Shi M, Guo WP, Wang MR, et al. Identification of novel and diverse rotaviruses in rodents and insectivores, and evidence of cross-species transmission into humans. *Virology.* 2016;494:168-177. Doi: 10.1016/j.virol.2016.04.017
21. Rotavirus Classification Working Group (RCWG). Newly assigned genotypes. 2017.
22. Fields B N, KNIPE DM and HOWLEY PM. *Fields virology*. Philadelphia, Wolters Kluwer Health/Lippincott Williams & Wilkins. 2013.
23. Shetty SA, M Mathur and JM Deshpande. Complete genome analysis of a rare group A rotavirus, G11P[25], isolated from a child in Mumbai, India, reveals interspecies transmission and reassortment with human rotavirus strains. *J Med Microbiol.* 2014;63(Pt 9):1220-1227. Doi: 10.1099/jmm.0.070524-0
24. Matthijnssens J and M Van Ranst. Genotype constellation and evolution of group A rotaviruses infecting humans. *Curr Opin Virol.* 2012;2(4):426-433. Doi: 10.1016/j.coviro.2012.04.007
25. Ward RL, Bernstein DI, Young EC, Sherwood JR, Knowlton DR, Schiff GM. Human rotavirus studies in volunteers: determination of infectious dose and serological response to infection. *J Infect Dis.* 1986;154(5):871-880.
26. Kraft RO and WJ Fry. Operative technic of selective gastric vagotomy. *Am J Surg.* 1963;105:423-435.
27. Ray PG, Kelkar SD, Walimbe AM, Biniwale V, Mehendale S. Rotavirus immunoglobulin levels among Indian mothers of two socio-economic groups and occurrence of rotavirus infections among their infants up to six months. *J Med Virol.* 2007;79(3):341-349.
28. Boshuizen JA, Reimerink JH, Korteland-van Male AM, van Ham VJ, Koopmans MP, Büller HA, Dekker J, et al. Changes in small intestinal homeostasis, morphology, and gene expression during rotavirus infection of infant mice. *J Virol.* 2003;77(24):13005-13016.
29. Greenberg HB and MK Estes. Rotaviruses: from pathogenesis to vaccination. *Gastroenterology.* 2009;136(6):1939-1951.
30. Lekana-Douki SE, Kombila-Koumavor C, Nkoghe D, Drosten C, Drexler JF, Leroy EM. Molecular epidemiology of enteric viruses and genotyping of rotavirus A, adenovirus and astrovirus among children under 5 years old in Gabon. *Int J Infect Dis.* 2015;34:90-95. Doi: 10.1016/j.ijid.2015.03.009
31. Estes MK and AP Morris. A viral enterotoxin. A new mechanism of virus-induced pathogenesis. *Adv Exp Med Biol.* 1999;473:73-82.
32. Lundgren O, Peregrin AT, Persson K, Kordasti S, Uhnöo I, Svensson L. Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science.* 2000;287(5452):491-495.
33. Aristegui J, Ferrer J, Salamanca I, Garrote E, Partidas A, San-Martin 5, San-Jose B, et al. Multicenter prospective study on the burden of rotavirus gastroenteritis in children less than 3 years of age in Spain. *BMC Infect Dis.* 2016;16(1):549.
34. Luchs A, Cilli A, Morillo SG, de Cassia Compagnoli Carmona R, do Carmo Sampaio Tavares Timenetsky M. Rotavirus in adults, Brazil, 2004-2011: G2P[4] dominance and potential impact on vaccination. *Braz J Infect Dis.* 2014;18(1):53-59. Doi: 10.1016/j.bjid.2013.05.010
35. Azemi M, Berisha M, Ismaili-Jaha V, Kolgeci S, Avdiu M, Jakupi X, Hoxha R, et al. Socio-demographic, Clinical and Laboratory Features of Rotavirus Gastroenteritis in Children Treated in Pediatric Clinic. *Mater Sociomed.* 2013;25(1):9-13.
36. Dennehy PH. Transmission of rotavirus and other enteric pathogens in the home. *Pediatr Infect Dis J.* 2000;19(10 Suppl):S103-105.
37. Johansen K, Hedlund KO, Zweyberg-Wirgart B, Bennet R. Complications attributable to rotavirus-induced diarrhoea in a Swedish paediatric population: report from an 11-year surveillance. *Scand J Infect Dis.* 2008;40(11-12):958-964. Doi: 10.1080/00365540802415509
38. Li B, Ding S, Feng N, Mooney N, Ooi YS, Ren L, Diep J, et al. Drebrin restricts rotavirus entry by inhibiting dynamin-mediated endocytosis. *Proc Natl Acad Sci U S A.* 2017;114(18):E3642-e3651. Doi: 10.1073/pnas.1619266114
39. Trask SD and PR Dormitzer. Assembly of highly infectious rotavirus particles recoated with recombinant outer capsid proteins. *J Virol.* 2006;80(22):11293-11304.
40. Fajardo T, Sung PY, Celma CC, Roy P. Rotavirus Genomic RNA Complex Forms via Specific RNA-RNA Interactions: Disruption of RNA Complex Inhibits Virus Infectivity. *Viruses.* 2017;9(7):E167. Doi: 10.3390/v9070167
41. Pastor AR, Rodríguez-Limas WA, Contreras MA, Esquivel E, Esquivel-Guadarrama F, Ramírez OT, Palomares LA, et al. The assembly conformation of rotavirus VP6 determines its protective efficacy against rotavirus challenge in mice. *Vaccine.* 2014;32(24):2874-2877. Doi: 10.1016/j.vaccine.2014.02.018
42. Gardet A, Breton M, Fontanges P, Trugnan G, Chwetzoff S. Rotavirus spike protein VP4 binds to and remodels actin bundles of the epithelial brush border into actin bodies. *J Virol.* 2006;80(8):3947-3956.
43. Richardson S, Grimwood K, Gorrell R, Palombo E, Barnes G, Bishop R. Extended excretion of rotavirus after severe diarrhoea in young children. *Lancet.* 1998;351(9119):1844-1848.
44. Parashar UD, EA Nelson and G Kang. Diagnosis, management, and prevention of rotavirus gastroenteritis in children. *Bmj.* 2013;347:f7204. Doi: 10.1136/bmj.f7204
45. A J Herring, N F Inglis, C K Ojeh, D R Snodgrass, J D Menzies. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. *J Clin Microbiol.* 1982;16(3):473-477.
46. E E Thomas, M L Puterman, E Kawano, M Curran. Evaluation of seven immunoassays for detection of rotavirus in pediatric stool samples. *J Clin Microbiol.* 1988;26(6):1189-1193.
47. Iturriza-Gómara M, G Kang and J Gray. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. *J Clin Virol.* 2004;31(4):259-265.

48. Hartling L, Bellemare S, Wiebe N, Russell K, Klassen TP, Craig W. Oral versus intravenous rehydration for treating dehydration due to gastroenteritis in children. *Cochrane Database Syst Rev*. 2006;(3):Cd004390.
49. Hahn S, S Kim and P Garner. Reduced osmolarity oral rehydration solution for treating dehydration caused by acute diarrhoea in children. *Cochrane Database Syst Rev*. 2002;(1):Cd002847.
50. Dalby-Payne JR and EJ Elliott. Gastroenteritis in children. *BMJ Clin Evid*. 2011.
51. Lee DK, Park JE, Kim MJ, Seo JG, Lee JH, Ha NJ. Probiotic bacteria, *B. longum* and *L. acidophilus* inhibit infection by rotavirus in vitro and decrease the duration of diarrhea in pediatric patients. *Clin Res Hepatol Gastroenterol*. 2015;39(2):237-244. Doi: 10.1016/j.clinre.2014.09.006
52. Gonzalez-Ochoa G, Flores-Mendoza LK, Icedo-Garcia R, Gomez-Flores R, Tamez-Guerra P. Modulation of rotavirus severe gastroenteritis by the combination of probiotics and prebiotics. *Arch Microbiol*. 2017;199(7):953-961.
53. Patel MM, Steele D, Gentsch JR, Wecker J, Glass RI, Parashar UD. Real-world impact of rotavirus vaccination. *Pediatr Infect Dis J*. 2011;30(1 Suppl):S1-5. Doi: 10.1097/INF.0b013e3181fefa1f
54. Jiang V, Jiang B, Tate J, Parashar UD, Patel MM. Performance of rotavirus vaccines in developed and developing countries. *Hum Vaccin*. 2010;6(7):532-542.
55. Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R, Meurice F, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet*. 2007;370(9601):1757-1763.
56. Rotavirus vaccines WHO position paper: January 2013 - Recommendations. *Vaccine*. 2013;31(52):6170-6171. Doi: 10.1016/j.vaccine.2013.05.037
57. Arif Mohammad Tanmoy , ASM Nawshad Uddin Ahmed , Rajesh Arumugam , Belal Hossain , Mahfuza Marzan , Shampa Saha , Shams El Arifeen, et al. Rotavirus Surveillance at a WHO-Coordinated Invasive Bacterial Disease Surveillance Site in Bangladesh: A Feasibility Study to Integrate Two Surveillance Systems. *PLoS One*. 2016;11(4):e0153582.
58. Linhares AC, Velázquez FR, Pérez-Schael I, Sáez-Llorens X, Abate H, Espinoza F, López P, et al. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. *Lancet*. 2008;371(9619):1181-1189. Doi: 10.1016/S0140-6736(08)60524-3
59. Michelle Gail Goveia, Z José Miguel Rodríguez, Michael J Dallas, Robbin F Itzler, John W Boslego, Penny M Heaton, Mark J Dinubile, et al. Safety and efficacy of the pentavalent human-bovine (WC3) reassortant rotavirus vaccine in healthy premature infants. *Pediatr Infect Dis J*. 2007;26(12):1099-1104.
60. Block SL, Vesikari T, Goveia MG, Rivers SB, Adeyi BA, Dallas MJ, Bauder J, et al. Efficacy, immunogenicity, and safety of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine at the end of shelf life. *Pediatrics*. 2007;119(1):11-18.
61. Afrad MH, Hassan Z, Farjana S, Moni S, Barua S, Das SK, Faruque AS, et al. Changing profile of rotavirus genotypes in Bangladesh, 2006-2012. *BMC Infect Dis*. 2013;13:320. Doi: 10.1186/1471-2334-13-320
62. Manish Patel, Andi L Shane, Umesh D Parashar, Baoming Jiang, Jon R Gentsch, Roger I Glass. Oral rotavirus vaccines: how well will they work where they are needed most? *J Infect Dis*. 2009;200(Suppl 1):S39-48. Doi: 10.1086/605035
63. Manish Patel, Andi L Shane, Umesh D Parashar, Baoming Jiang, Jon R Gentsch, Roger I Glass. Oral rotavirus vaccines: how well will they work where they are needed most? *The Journal of infectious diseases*. 2009;200(0-1):S39-S48.
64. Sirajuddin Ahmed, AK Siddique, Anwarul Iqbal, FKM Nurur Rahman, Md Noor Islam, Arif Sobhan, Md Rafiqul Islam, RB Sack. Causes for hospitalizations at upazila health complexes in Bangladesh. *J Health Popul Nutr*. 2010;28(4):399-404.
65. Tanaka G, Faruque AS, Luby SP, Malek MA, Glass RI, Parashar UD. Deaths from rotavirus disease in Bangladeshi children: estimates from hospital-based surveillance. *Pediatr Infect Dis J*. 2007;26(11):1014-1018.
66. Paul SK, Kobayashi N, Nagashima S, Ishino M, Watanabe S, Alam MM, Ahmed MU, et al. Phylogenetic analysis of rotaviruses with genotypes G1, G2, G9 and G12 in Bangladesh: evidence for a close relationship between rotaviruses from children and adults. *Arch Virol*. 2008;153(11):1999-2012. Doi: 10.1007/s00705-008-0212-9
67. News report on the daily star. <https://www.thedailystar.net/business/news/capita-income-hits-1909-1717606>. 2019.