SPECIAL ARTICLE

Rotavirus in India: Forty Years of Research

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Rotavirus was first identified as a human pathogen just over 40 years ago, and work on this pathogen in India started shortly thereafter. Subsequent studies have confirmed its pre-eminent role in gastroenteritis in children in India. Standardized surveillance has enabled the documentation of the high burden of disease, and has demonstrated that there is considerable geographic and temporal variation in strain circulation. Internationally licensed vaccines, vaccine candidates based on indigenous strains and out-licensed strains have been tested for safety, immunogenicity and efficacy; three vaccines are now licensed in India and are used in the private sector. Public sector vaccination has begun, and it will be path-breaking for Indian vaccinologists to measure impact of vaccine introduction in terms of safety and effectiveness. So far, India has kept pace with international epidemiologic and vaccine research on rotavirus, and these efforts should continue.

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In 1973, Ruth Bishop and colleagues published a paper in the Lancet describing virus particles in epithelial cells of the duodenal mucosa of children with acute non-bacterial gastroenteritis [1]. Two years later, Ian Holmes, the electron microscopist – who had seen the round-wheel shaped structures in the samples that Bishop provided – visited Southern India and taught Minnie Mathan at the Christian Medical College, Vellore how to recognize these distinctive viruses. The first paper on rotavirus from India was published in 1977 [2] that described the virus to be associated with 26% of severe gastroenteritis. Shortly thereafter, Dr. Panicker in Calicut (as it was then known), contacted Dr. Mathan to analyze samples from an outbreak of gastroenteritis, and their joint work demonstrated that rotavirus was the cause of the outbreak and subsequently that rotavirus disease was seasonal [3].

Scientists learnt that it was possible to distinguish rotaviruses based on the patterns of migration of the 11 segments of double-stranded RNA. Electropherotyping methods were established that showed different circulating types of rotavirus, with variations by location. Subsequently, enzyme immunoassays become available, and several researchers in India began to identify rotavirus infections not only in children with acute diarrhea, but also in animals [4,5]. Bhan, *et al*. [6] from All India Institute of Medical Sciences (New Delhi) showed that a large proportion of neonates in their nursery were asymptomatically infected with rotavirus. When these babies were followed up over time, it was shown that these children were protected from severe rotavirus gastroenteritis, and that the strains isolated from these children were all similar based on electropherotyping [6]. Dr. Bhan, who later became the Secretary of the Department of Biotechnology (DBT; Government of India), collaborated with Roger Glass, whom he had met when Dr. Glass had worked in Bangladesh. Dr. Glass went on to the Centers of Disease Control and Prevention in Atlanta, and supported the characterization of the 116E neonatal strain that had been isolated in AIIMS, by Dr Bimal Das. His work, based on sequencing of the strain, showed that the strain was unusual, in belonging to the G9P[11] serotype, because most strains detected up to that time from humans had been G1-4 and in being a natural reassortant strain, carrying the P[11] gene of bovine origin [7]. Further studies explored why neonates were infected when mothers had transferred anti-rotavirus antibodies to their infants. It was shown that the infected children did mount an antibody response, and it was postulated that the presence of the bovine capsid protein allowed the children to get infected even though transplacental or breast milk antibodies were received from the mother [8].

A similar story emerged in Bangalore, where C Durga Rao and his colleagues identified a strain that asymptomatically infected neonates resulting in subsequent decreases in rotavirus infection and disease. The strain, called I321, was also a bovine human reassortant, but unlike 116E, which has only one bovine gene, it consisted of mainly bovine genes [9].

While these studies were being done, through the 1980s and early 1990s, various enzyme immunoassays

and electrophoresis techniques were used to identify rotaviruses from children in out-patient and in-patient settings, and wherever studies were done, rotaviruses were associated with a significant proportion of acute diarrheal disease, up to 20-50% with winter peaks, particularly in the North [10]. The enzyme immunoassay kits were expensive and the National Institute of Virology developed reagents for a similar test for rotavirus [11], but it was not widely used, because testing for rotavirus in routine practice was non-existent. Specific sera for typing of the two outer capsid proteins became available through international collaborations and the diversity of rotaviruses in India and the change in strains was increasingly evident. When polymerase chain reaction (PCR)-based techniques were well established in the 1990s, they confirmed the finding of high diversity and the occurrence of unusual strains, possibly due to zoonotic infections [12].

In parallel to the several small surveillance studies, the two neonatal strains of rotavirus that had been identified in Delhi and Bangalore were adapted to cell culture and grown to make vaccine candidates. The DBT (India) and the National Institutes of Health (US) supported rotavirus vaccine development through the Indo-US Vaccine Action Program (VAP) that was established in the 1980s through several grants. In the late 1990s, the VAP decided to support a new company, Bharat Biotech International Limited, to take the development of 116E and I321 vaccines forward. Initial phase I testing had been conducted in the US with support from the CDC and NIH, but the studies were repeated in India and extended into phase II [9]. In phase II, the I321 strain was found to be less immunogenic with only 30% of children seroconverting, whereas the 116E strain seroconverted more than 80% of children, and hence only the 116E strain was taken forward into phase III. Other studies in neonates had shown that strains, that resembled I321, infected children in Vellore, and that these children were not protected from subsequent rotavirus infection or diarrhea [13]. While the indigenous vaccine candidate was undergoing clinical testing, the Indian Council for Medical Research (ICMR) decided to make a large investment in rotavirus surveillance and established a multi-site network, which unlike several previous studies that had all differed in study design and diagnostic approaches, used similar methods for recruitment and testing. This standardized approach revealed that unlike previous studies which had estimated that rotavirus caused about 20% of hospitalized gastroenteritis, the proportion that were testing positive was closer to 40% [12]. In addition to the studies focused on burden of disease and vaccination, Indian researchers initiated more basic studies on the biology of rotavirus – studying structure and function of rotaviral proteins, thus complementing the work that is being conducted in other settings [14,15].

Despite the basic research and the multitude of hospital-based studies, there have been very few community-based studies on rotavirus in India. The largest birth cohort study to evaluate rotavirus infection was conducted in Vellore between 2001 and 2006 [16, 17]. This study showed that unlike the previous birth cohort studies in other parts of the world – although rotavirus infection was common and rotavirus was the most important pathogen causing diarrhea in the community – the protection afforded by prior rotavirus infection was less than that seen in other birth cohorts [16]. This led to the question of how well vaccines would work, and modelling studies based on the Vellore data estimated a protection of about 50% in disadvantaged populations [18].

While the indigenous vaccine candidate was in phase II and III studies, the two internationally licensed vaccines underwent immunogenicity bridging studies at multiple sites in India. Based on 58% immunogenicity for Rotarix and 83% for Rotateq – but by different ways of assessing immunogenicity – Rotarix and Rotateq, were licensed [19, 20], and used in the private market, with the Indian Academy of Pediatrics, reviewing their performance and making recommendations for their use [21]. In 2014, the results of the efficacy trial with 116E became available, and at 55% efficacy, the performance of this vaccine was comparable to that of Rotarix and Rotateq in Africa and other countries in Asia [22]. This was despite the fact that the very close monitoring and early treatment of children in the efficacy trial considerably reduced the incidence of severe disease.

In parallel with the vaccine testing, a number of studies estimated the burden of disease in India and the cost-effectiveness of rotavirus vaccines, and all studies demonstrated that in India, the vaccines would be cost-effective at the price at which vaccines were available for the Indian private and public markets [23,24]. India's birth cohort of 27 million is the largest in the world, and unfortunately even though the number of diarrheal deaths is decreasing rapidly, the number of deaths attributed to rotavirus is numerically the largest for any country. Given that mortality due to diarrheal disease is decreasing with access to care, rehydration and better nutrition, the impact of vaccines should be measured not only as reduction in mortality but also in averted hospitalizations, as emphasized in an editorial in this issue [25]. There is also a need to revisit cost-effectiveness, since mortality

has decreased and costing studies which informed earlier estimates were collected a decade ago and excluded costs in children admitted with gastroenteritis who required higher levels of care, which are now available, along with limited more recent estimates [26,27].

The several studies by the ICMR and DBT as well as academic researchers in India over decades, resulted in a situation that when affordable rotavirus vaccines became available for the national immunization program, the evidence base for vaccine introduction and the cost utility of rotavirus vaccines already existed, and it was possible for the National Technical Advisory Group on Immunization to recommend to the Ministry of Health and Family Welfare that the vaccine should be introduced for the children who need it the most. The recommendation was accepted and a phased introduction began in 2016 with Odisha, Andhra Pradesh, Haryana and Himachal Pradesh, but will roll out nation-wide as supply becomes available for the rest of India.

Other than for polio, which was a global eradication effort, and hence different from rotavirus, there have been few systematic efforts to assess the impact of a newly introduced vaccine in India. In countries where vaccines have been introduced nation-wide, there have been remarkable effects of reduction in severe rotavirus gastroenteritis, all-cause gastroenteritis and all-cause gastroenteritis mortality as well as reductions in gastroenteritis in unvaccinated age groups, indicating a herd effect [28]. Such studies are planned for India which will assess the effectiveness of the vaccine in routine use as well as monitor its safety [25,29]. Several concerns have been raised in the media about the safety of rotavirus vaccines and the potential for intussusception. Both Rotarix and Rotateq have been associated with a small increased risk where they have been given to several hundreds of thousands of children [28]. Rotavac, the vaccine that will, at least, initially, be used in the public health immunization system in India, has not been tested in such large numbers, and while the studies conducted so far have shown no risk, there need to be continued monitoring both through the post-marketing surveillance required by the Drugs Controller General of India as well as in the public health immunization system.

The ICMR has been preparing for the monitoring of impact through expanded surveillance, which shows that the burden continues to be high [30,31], and similar studies have also been conducted by other researchers across India [32-35]. While the epidemiologic and vaccine studies were conducted during the past decade, there were also efforts to understand the basis of the immune response to rotavirus and rotavirus vaccines

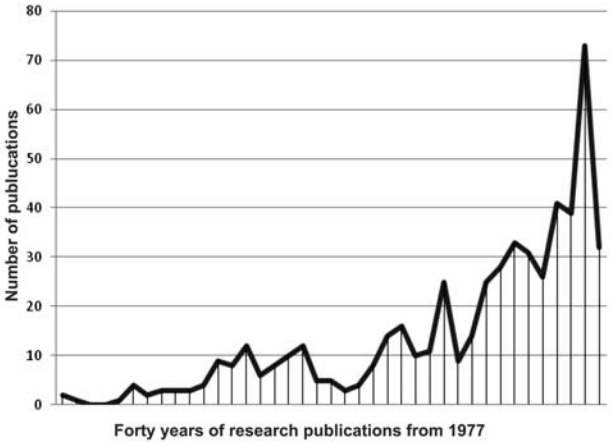

FIG. 1 *Peer-reviewed publications on rotavirus from India.*

[36, 37] and the reasons why rotavirus vaccination efficacy was less in developed than in developing countries. Several reasons have been proposed, including high levels of maternal antibodies, environmental enteropathy, and malnutrition or micronutrient deficiencies. Studies are being conducted on approaches to improve performance of vaccines, but with little success so far [38, 39]. One question that remains unanswered is how well rotavirus vaccines perform in children of upper socio-economic status in India, and such a study has never been done.

Other companies in India are also working on rotavirus vaccines, with Serum Institute of India, Shantha Biotechnics and Hilleman Laboratories all having rotavirus vaccine programs at various stages of development. Overall, rotavirus has been one vaccine preventable disease where India has kept pace with the rest of the world in conducting comprehensive research, with over 500 studies resulting in publications in peer-reviewed journals (***Fig.* 1**). We have now developed at least one indigenous vaccine, and whether it is this vaccine or others that are used, we should ensure that we continue to conduct appropriate research to monitor this important cause of childhood gastroenteritis, its treatment and prevention.

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REFERENCES

1. Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Detection of a new virus by electron microscopy of faecal extracts from children with acute gastroenteritis. Lancet. 1974;1:149-51.
2. Maiya PP, Pereira SM, Mathan M, Bhat P, Albert MJ, Baker SJ. Aetiology of acute gastroenteritis in infancy and early childhood in southern India. Arch Dis Child. 1977;52:482-5.

3. Paniker CK, Mathew S, Dharmarajan R, Mathan MM, Mathan VI. Epidemic gastroenteritis in children associated with rotavirus infection. Indian J Med Res. 1977;66:525-9.
4. Hrdy DB. Rotavirus antibodies in hanuman langurs (*Presbytis entellus*). J Med Primatol. 1982;11:35-8.
5. Singh A, Pandey R. Analysis of electrophoretypes of rotavirus from diarrhoeic faeces of neonatal buffalo calves in India. Acta Virol. 1988;32:156-9.
6. Bhan MK, Lew JF, Sazawal S, Das BK, Gentsch JR, Glass RI. Protection conferred by neonatal rotavirus infection against subsequent rotavirus diarrhea. J Infect Dis. 1993;168:282-7.
7. Cunliffe NA, Das BK, Ramachandran M, Bhan MK, Glass RI, Gentsch JR. Sequence analysis demonstrates that VP6, NSP1 and NSP4 genes of Indian neonatal rotavirus strain 116E are of human origin. Virus Genes. 1997;15:39-44.
8. Ramachandran M, Vij A, Kumar R, Das BK, Gentsch JR, Bhan MK, *et al*. Lack of maternal antibodies to P serotypes may predispose neonates to infections with unusual rotavirus strains. Clin Diagn Lab Immunol. 1998;5:527-30.
9. Glass RI, Bhan MK, Ray P, Bahl R, Parashar UD, Greenberg H, *et al*. Development of candidate rotavirus vaccines derived from neonatal strains in India. J Infect Dis. 2005 ;192(Suppl 1):S30-5.
10. Ramani S, Kang G. Burden of disease and molecular epidemiology of group A rotavirus infections in India. Indian J Med Res. 2007;125:619-32.
11. Kelkar SD, Bhide VS, Ranshing SS, Bedekar SS. Rapid ELISA for the diagnosis of rotavirus. Indian J Med Res. 2004;119:60-5.
12. Kang G, Desai R, Arora R, Chitamabar S, Naik TN, Krishnan T, *et al*. Diversity of circulating rotavirus strains in children hospitalized with diarrhea in India, 2005-2009. Vaccine. 2013;31:2879-83.
13. Banerjee I, Gladstone BP, Le Fevre AM, Ramani S, Iturriza-Gomara M, Gray JJ, *et al*. Neonatal infection with G10P[11] rotavirus did not confer protection against subsequent rotavirus infection in a community cohort in Vellore, South India. J Infect Dis. 2007;195:625-32.
14. Rajasekaran D, Sastri NP, Marathahalli JR, Indi SS, Pamidimukkala K, Suguna K, *et al*. The flexible C terminus of the rotavirus non-structural protein NSP4 is an important determinant of its biological properties. J Gen Virol. 2008;89:1485-96.
15. Bhowmick R, Halder UC, Chattopadhyay S, Nayak MK, Chawla-Sarkar M. Rotavirus-encoded nonstructural protein 1 modulates cellular apoptotic machinery by targeting tumor suppressor protein p53. J Virol. 2013;87:6840-50.
16. Gladstone BP, Ramani S, Mukhopadhya I, Muliyil J, Sarkar R, Rehman AM, *et al*. Protective effect of natural rotavirus infection in an Indian birth cohort. N Engl J Med. 2011;365:337-46.
17. Sarkar R, Gladstone BP, Warier JP, Sharma SL, Raman U, Muliyil J, *et al*. Rotavirus and other diarrheal disease in a birth cohort from Southern Indian community. Indian Pediatr. 2016:53:583-8.
18. Lopman BA, Pitzer VE, Sarkar R, Gladstone B, Patel M, Glasser J, *et al*. Understanding reduced rotavirus vaccine efficacy in low socio-economic settings. PLoS One. 2012;7:e41720.
19. Narang A, Bose A, Pandit AN, Dutta P, Kang G, Bhattacharya SK, *et al*. Immunogenicity, reactogenicity and safety of human rotavirus vaccine (RIX4414) in Indian infants. Hum Vaccin. 2009;5:414-9.
20. Lokeshwar MR, Bhave S, Gupta A, Goyal VK, Walia A. Immunogenicity and safety of the pentavalent human-bovine (WC3) reassortant rotavirus vaccine (PRV) in Indian infants. H um Vaccin Immunother. 2013;9:172-6.
21. Vashishtha VM, Choudhury P, Kalra A, Bose A, Thacker N, Yewale VN, *et al*. Indian Academy of Pediatrics. Indian Academy of Pediatrics (IAP) recommended immunization schedule for children aged 0 through 18 years—India, 2014 and updates on immunization. Indian Pediatr. 2014;51:785-800.
22. Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, *et al*. India Rotavirus Vaccine Group. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: A randomised, double-blind, placebo-controlled trial. Lancet. 2014;383:2136-43.
23. Megiddo I, Colson AR, Nandi A, Chatterjee S, Prinja S, Khera A, *et al*. Analysis of the Universal Immunization Programme and introduction of a rotavirus vaccine in India with IndiaSim. Vaccine. 2014;32 Suppl 1:A151-61.
24. Esposito DH, Tate JE, Kang G, Parashar UD. Projected impact and cost-effectiveness of a rotavirus vaccination program in India, 2008. Clin Infect Dis. 2011;52:171-7.
25. Arora R, Swaminathan S. Ready to measure impact? The introduction of rotavirus vaccine in India. Indian Pediatr. 2016;53:565-7.
26. Mathew A, Srinivasan R, Venugopal S, Kang G. Direct medical costs in children with rotavirus and non-rotavirus diarrhea admitted to a pediatric intensive care unit and high dependency unit in Delhi. Indian Pediatr. 2016;53:639-41.
27. Jacob J, Joseph TK, Srinivasan R, Kompithra RZ, Simon A, Kang G. Direct and indirect costs of pediatric gastroenteritis in Vellore, India. Indian Pediatr. 2016;642-4.
28. Rao TS, Arora R, Khera A, Tate JE, Parashar U, Kang G; Indian Rotavirus Vaccine Working Group. Insights from global data for use of rotavirus vaccines in India. Vaccine. 2014;32(Suppl 1):A171-8.
29. Mathew MA, Venugopal S, Arora R, Kang G. Leveraging the National Rotavirus Surveillance Network for monitoring intussusception. Indian Pediatr. 2016;53:635-8.
30. Mehendale S, Venkatasubramanian S, Girish Kumar CP, Kang G, Gupte MD, Arora R. Expanded Indian National Rotavirus Surveillance Network in the context of rotavirus vaccine introduction. Indian Pediatr. 2016;53:575-81.
31. Girish Kumar CP, Venkatasubramanian S, Kang G, Arora R, Mehendale S, for the National Rotavirus Surveillance Network. Profile and trends of rotavirus gastroenteritis in under-five children in India (2012-2014): Preliminary report of the Indian National Rotavirus Surveillance Network. Indian Pediatr. 2016;53:619-22.
32. Kumar A, Basu S, Vashishtha VM, Choudhury P. Burden of rotavirus diarrhea in under-five Indian children. Indian Pediatr. 2016;53:607-17.
33. Teotia N, Upadhyay A, Agarwal S, Garg A, Shah D.

Rotavirus diarrhea in children presenting to an urban hospital in western Uttar Pradesh, India. Indian Pediatr. 2016;53:627-9.
34. Maher G, Pradhan G, Shetty S, Ranshing S, Dample A, Chitambar S. Rotavirus infection in children with acute gastroenteritis in Aurangabad, central Maharashtra. Indian Pediatr. 2016;53:631-3.
35. Gupta M, Singh MP, Guglani V, Mahajan KS. Hospital-based surveillance of rotavirus diarrhea among under-five children in Chandigarh. Indian Pediatr. 2016;53:651-2.
36. Paul A, Babji S, Sarkar R, Lazarus RP, Kang G. Rotavirus-specific salivary and fecal IgA in Indian children and adults. Indian Pediatr. 2016;53:601-6.
37. Premkumar P, Lopman B, Ramani S, Paul A, Gladstone B, Muliyil J, *et al.* Association of serum antibodies with protection against rotavirus infection and disease in South Indian children. Vaccine 2014;32(Suppl 1);A55-61.
38. Rongsen-Chandola T, Strand TA, Goyal N, Flem E, Rathore SS, Arya A, *et al*. Effect of withholding breastfeeding on the immune response to a live oral rotavirus vaccine in North Indian infants. Vaccine. 2014;32(Suppl 1):A134-9.
39. Kompithra RZ, Paul A, Manoharan D, Babji S, Sarkar R, Mathew LG, *et al*. Immunogenicity of a 3 dose and 5 dose oral human rotavirus vaccine (RIX4414) schedule in south Indian infants. Vaccine. 2014;32;(Suppl 1):A129-33.