RESEARCH PAPER

Expanded Indian National Rotavirus Surveillance Network in the Context of Rotavirus Vaccine Introduction

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Objective: To extend a nation-wide rotavirus surveillance network in India, and to generate geographically representative data on rotaviral disease burden and prevalent strains.

Design: Hospital-based surveillance.

Setting: A comprehensive multicenter, multi-state hospital based surveillance network was established in a phased manner involving 28 hospital sites across 17 states and two union territories in India.

Patients: Cases of acute diarrhea among children below 5 years of age admitted in the participating hospitals.

Results: During the 28-month study period between September 2012 and December 2014, 11898 children were enrolled and stool samples from 10207 children admitted with acute diarrhea were tested; 39.6% were positive for rotavirus. Highest positivity was seen in Tanda (60.4%) and Bhubaneswar (60.4%) followed by Midnapore (59.5%). Rotavirus infection was seen more among children aged below 2 years with highest (46.7%) positivity in the age group of 12-23 months. Cooler months of September – February accounted for most of the rotavirus-associated gastroenteritis, with highest prevalence seen during December – February (56.4%). 64% of rotavirus-infected children had severe to very severe disease. G1 P[8] was the predominant rotavirus strain (62.7%) during the surveillance period.

Conclusions: The surveillance data highlights the high rotaviral disease burden in India. The network will continue to be a platform for monitoring the impact of the vaccine.

Keywords: *Epidemiology; Rotavirus diarrhea, Vaccine.*

Diarrheal diseases account for 1 in 10 child deaths worldwide, making diarrhea the second leading cause of deaths among children under the age of 5 years [1]. Rotavirus infections are the most common cause of severe gastroenteritis in children below 5 years of age worldwide and account for 5% of all deaths among children in this age group [2]. Nearly 453,000 (420,000 – 494,000) child deaths were estimated to have occurred globally during 2008 due to rotavirus infection with 22% in children below five years of age in India alone [3]. Recent estimates have shown that about 872,000 hospitalizations and 78,500 deaths occur due to rotavirus infections annually in India [4].

Several studies on rotavirus disease have been conducted across different parts of India but due to differences in study design, operational definitions, populations examined, recruitment strategies, and detection systems used [5]; information on the national level on rotavirus diarrhea disease burden is not available.

The 'National Rotavirus Surveillance Network' (NRSN) was established in December 2005 by the Indian Council of Medical Research (ICMR) to evolve a sustainable surveillance platform to estimate and monitor the disease burden in children under 5 years of age hospitalized for diarrhea [5,6]. In order to make informed decisions regarding possible phased introduction of rotavirus vaccine in the country as part of the national immunization program, the surveillance network was expected to generate data on rotavirus disease burden and monitoring trends over time across the country. Another objective was to generate data on molecular epidemiology of rotavirus in India. This network generated valuable data for the period of 2005-2009. The first round of the NRSN (2005- 2009) was initiated with four laboratories and ten hospitals in seven different regions of India (***Fig.* 1**). The detailed results have been published earlier [5,6]. The analysis of surveillance data involving over 7000 children demonstrated both the high prevalence of rotavirus disease in India as well as the circulation of a diverse range of rotavirus strains [6]. Another notable finding was the emergence of G12 strains, particularly G12 P[6] strains, in both the Western and Northern region [5,6].

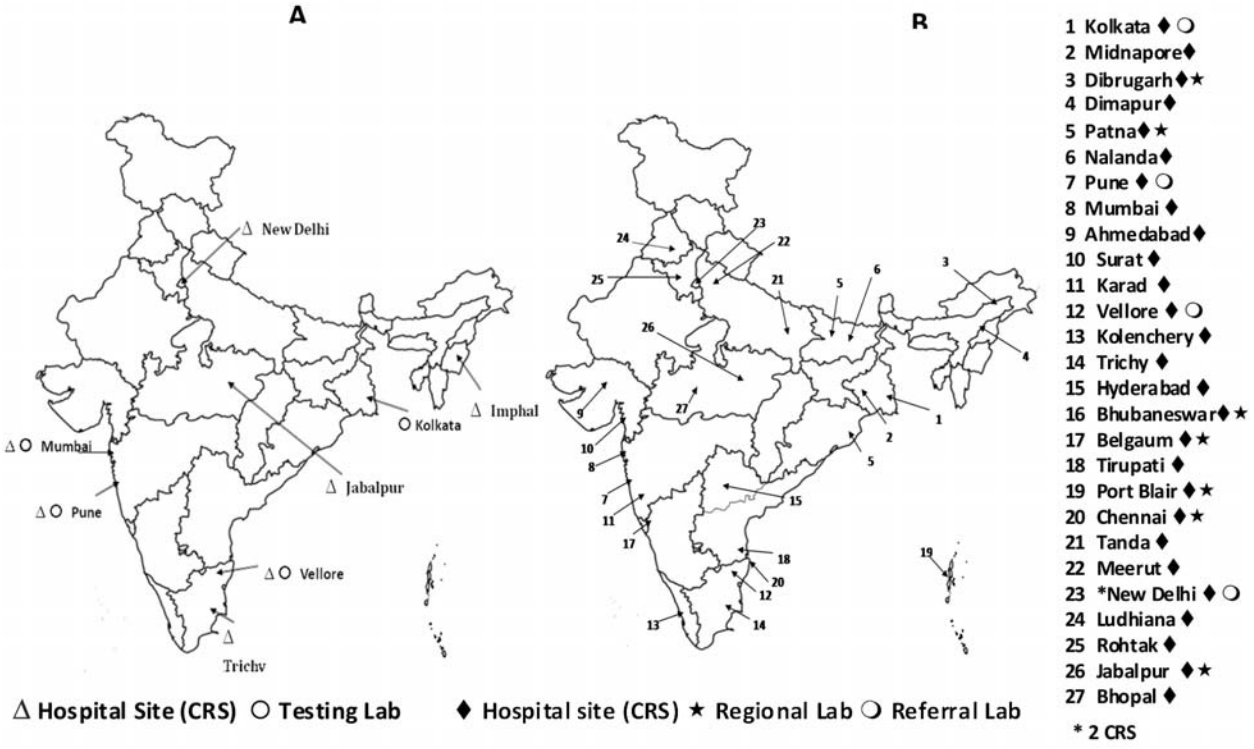

FIG. 1 *Hospital sites and testing laboratories (a) 2005 -2009 (b) 2012-2016*.

In November 2011, the expert advisory group set up by ICMR recommended expansion to include more clinical surveillance sites in hospitals and testing laboratories network to have more complete regional representation of the country. Following this recommendation, the NRSN was expanded to generate pan-India data on rotavirus disease burden. The present paper describes the process, methodology and framework of the expanded network (2012 onwards), and highlights the key findings and lessons learnt in this endeavor. Detailed analysis of the surveillance data is published in a separate paper.

METHODS

The Expanded National Rotavirus Surveillance Network (ENRSN) was planned to conduct hospital-based surveillance for rotavirus from 2009 onwards across the country. The network includes four Referral and seven Regional laboratories in Southern, Western, Northern and Eastern/ North-Eastern regions of the country, and 28 clinical recruitment sites (CRS) of hospitals in 17 states and two Union Territories with inpatient pediatric facilities that contribute clinical data and samples. The present ENRSN platform has been built with three phases of expansion in different parts of India. In Phase-1, the surveillance was initiated in September 2012 at 8 sites *viz*. Vellore (Tamil Nadu), Trichy (Tamil Nadu), Hyderabad (Telangana), Tirupati (Andhra Pradesh), Ludhiana (Punjab), Kolenchery (Kerala), New Delhi and Port Blair (Andaman & Nicobar). In September-October 2013, CRS in 12 cities *viz*. Kolkata (West Bengal), Midnapore (West Bengal), Pune (Maharasthra), Mumbai (Maharasthra), Karad (Maharasthra), Tanda (Himachal Pradesh), Meerut (Uttar Pradesh), New Delhi, Rohtak (Haryana), Dibrugarh (Assam), Dimapur (Nagaland), Belgaum (Karnataka), Surat (Gujarat) and Ahmedabad (Gujarat) initiated surveillance as part of the Phase-2 expansion. In Phase-3, additional sites [Patna (Bihar), Nalanda (Bihar), Bhubaneswar (Odisha), Chennai (Tamil Nadu), Jabalpur (Madhya Pradesh) and Bhopal (Madhya Pradesh)] started enrolling children in surveillance in July - August 2014. The network has been formalized by signing Memorandum of Understanding (MoUs) between each CRS, affiliated referral or regional laboratory and the coordinating center. The list of participating investigators and centers is provided as ***Annexure* 1.**

The Epidemiology and Communicable Diseases (ECD) Division of ICMR is responsible for administrative coordination of the network, providing funding and

leadership to the network. The National Institute of Epidemiology is the coordinating centers for this surveillance network involving 28 CRS across the country, and is responsible for selecting and initiating CRS, training in field component of the study protocol and data management. Its additional responsibility includes coordination, monitoring and evaluation as well as quality control of the clinical component, sample transfers, data management and analysis. Christian Medical College (CMC), Vellore is the coordinating laboratory that conducts training of laboratory personnel for laboratory procedures, designs and implements laboratory protocols and ensures laboratory quality management at all levels.

Clinical/Field Component

Site selection and support: Clinical recruitment sites (CRS) were identified by the National Institute of Epidemiology (NIE) with the help of partner institutes (referral and regional laboratories) in the respective regions *viz*. South, West, North and East/ North-East.

Establishing surveillance: The sanctioning and funding of each CRS and partner laboratories involved completion of specific documentation and submission to ICMR. The documentation process included signing of a MoU/ undertaking and obtaining clearance from the Ethics committees of the concerned hospitals / institutes.

Patient enrolment: All cases of acute diarrhea (≥3 loose stools in a 24 hour period) and of duration not greater than 5 days among children aged (0-59 months) admitted to the CRS for supervised oral or intravenous rehydration were considered for enrollment under ENRSN. Eligible children were enrolled after obtaining written informed consent from the parents/guardians. Each CRS enrolled children throughout the year, collected clinical data including history of rotavirus vaccination on a standardized clinical recruitment form/case report form (CRF), and also collected stool samples. Records such as date of admission for diarrhea management and enrolment were recorded in a diarrhea hospitalization logbook maintained in each CRS.

Sample collection: Whole stool specimen (~5ml) was collected from each child enrolled in the study and transported within 2 hours to the testing laboratory or stored at 4^0C until transportation. Samples stored at 4^0C after collection were transported in boxes with ice packs at weekly or fortnightly intervals to the testing laboratory.

Laboratory Testing

Stool samples were subjected to rotavirus screening using a commercial enzyme immunoassay (Premier Rotaclone, Meridian Biosciences) and genotyping for VP7 (G-typing) and VP4 (P-typing) by Reverse-transcription polymerase chain reaction (RT-PCR) [5]. Results of the laboratory analyses were recorded in a laboratory log book maintained in each testing laboratory.

Seven regional laboratories carried out ELISA for rotavirus antigen detection on stool samples collected at affiliated CRS. The regional laboratories were also responsible for entry of both clinical and laboratory data. Four referral laboratories carried out ELISA for screening on samples collected at CRS directly assigned to them, PCR for genotype characterization (including a subset of rotavirus positive samples from affiliated regional laboratories), and also completed data entry. Referral/ Regional Laboratories submitted monthly and quarterly reports. The organizational framework and data flow in the network is described in ***Fig.* 2**.

Training: Each referral and regional laboratory conducted hands-on training for staff recruited for the surveillance activity covering the three main components of the ENRSN program; namely, clinical, sample management, laboratory analysis and data entry.

Quality assurance: The coordinating laboratory at CMC, Vellore in coordination with NIE sent pretested panels of positive and negative samples to all the participating laboratories annually. Similarly CMC received set of ten consecutively tested samples for retesting from all the laboratories bi-annually. A panel of public health and child health experts constituted by ICMR in coordination with NIE carried out periodic monitoring visits to all levels of the network.

Data management: In the expanded round of NRSN a comprehensive, web-based data management system was introduced by NIE. The features of this system include double data entry, validation, innovative data matching interface, record level blocking and efficient data export to NIE, the Coordinating Center for ENRSN**.** The details of the data management framework have been described elsewhere [8]. Regional and referral laboratories were responsible for the data entry of the CRFs. Diarrheal disease severity was calculated using the Vesikari score [5]. Data validation and analysis was carried out at NIE.

Statistical analysis: Data were analyzed to assess the proportions of rotavirus-positive cases in terms of demographic factors, seasons, regions, disease severity and genotype distribution using SPSS 17.0.

RESULTS

During the 28-month study period from September 2012 to December 2014, a total of 14315 children with acute gastroenteritis were admitted to the participating

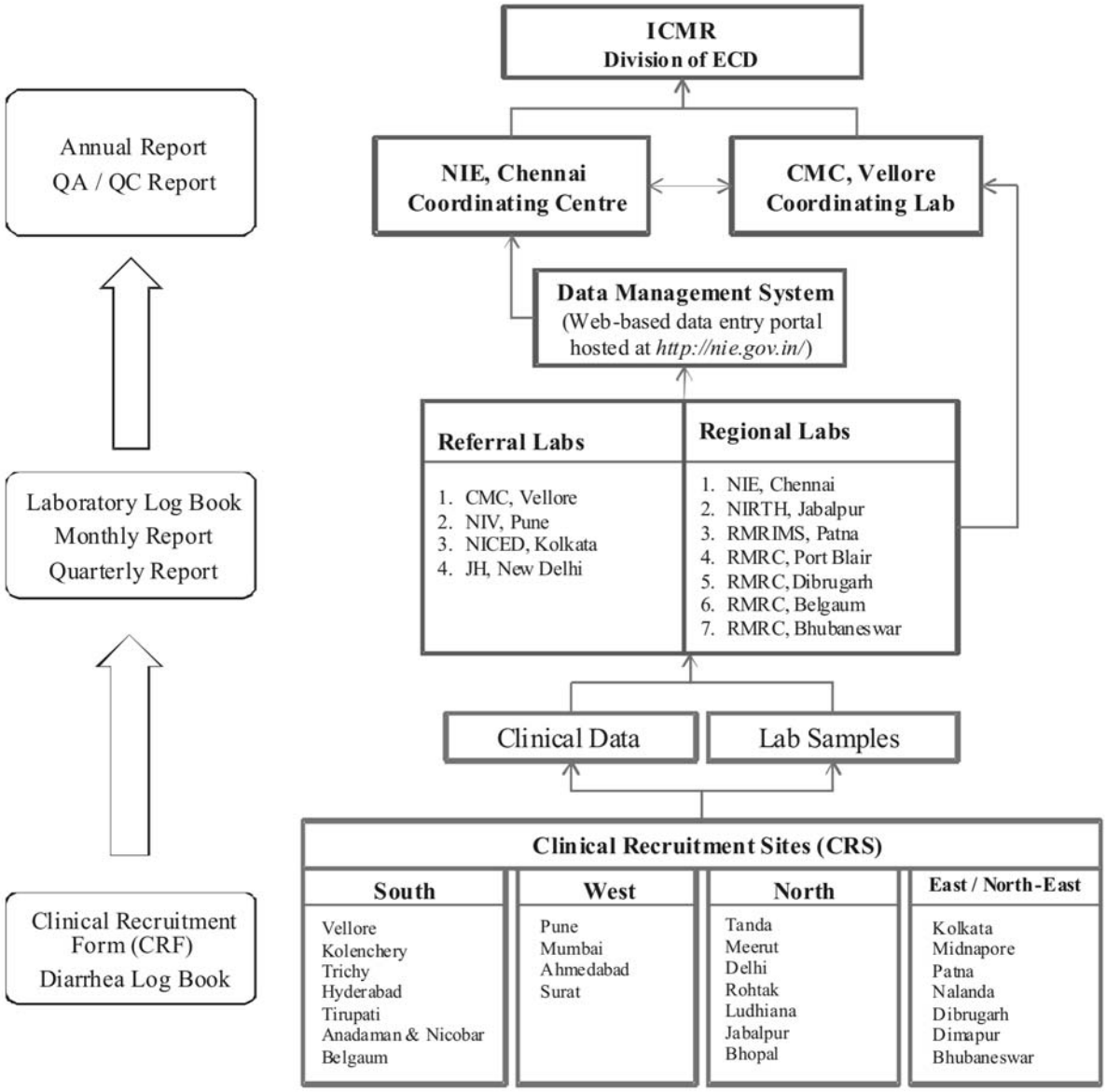

FIG. 2 *Sample and data flow in National Rotavirus Surveillance Network.*

hospitals and 11898 children were enrolled in to the surveillance. Among the 10207 children from whom stool samples were collected and analyzed, 39.6% were positive for rotavirus (***Table* I**). The highest rotavirus positivity was seen in Tanda (60.4%) and Bhubaneswar (60.4%), followed by Midnapore (59.5%). The lowest prevalence was observed in Nalanda (3.6%) and Patna (7.3%). Overall, most of the surveillance sites registered high proportion of rotavirus positivity.

Prevalence of rotavirus positivity in gastroenteritis was 38.1% (1890/4962) among children below one year of age; whereas the highest rotavirus disease burden was seen among children between 12-23 months (46.7%; 1514/3244). There was no significant difference in rotavirus positivity rates by gender. Pooled data analysis of seasonal prevalence showed that rotavirus infections were seen more commonly during the cooler months (September – February). The highest prevalence was observed during December – February (56.4%; 1668/2959) and lowest during June – August (20.6%; 461/2239). Diarrheal disease severity analysis among rotavirus-infected children revealed that 64% (2585/4042) of rotavirus infected children had severe or very

TABLE I REGIONAL AND SITE-WISE ROTAVIRUS POSITIVITY RATES OF CHILDREN HOSPITALIZED WITH ACUTE GASTROENTERITIS

Region	*CRS*	*Admitted/Enrolled*[*]	*Number of Samples Tested*[#]	*Rotavirus N(%) Positive*[#]
East	Kolkata	660/461	431	228 (52.9)
	Midnapore	918/602	592	352 (59.5)
	Bhubaneswar	710/411	268	162 (60.4)
	Dibrugarh	356/356	279	102 (36.6)
	Dimapur	326/326	260	117 (45.0)
	Patna	528/309	301	22 (7.3)
	Nalanda	244/230	169	6 (3.6)
	Total	3742/2695	2300	989 (43.0)
West	Pune	532/469	368	204 (55.4)
	Mumbai	453/415	369	133 (36.0)
	Ahmedabad	306/304	231	58 (25.1)
	Surat	540/508	424	169 (39.9)
	Karad	447/433	363	125 (34.4)
	Total	2278/2129	1755	689 (39.3)
South	Vellore	1088/957	789	240 (30.4)
	Kolenchery	790/709	529	239 (45.2)
	Trichy	331/324	282	141 (50.0)
	Hyderabad	756/410	388	93 (24.0)
	Tirupati	447/444	433	117 (27.0)
	Belgaum	290/280	255	80 (31.4)
	Port Blair	966/966	910	385 (42.3)
	Chennai	320/281	226	64 (28.3)
	Total	4988/4371	3812	1359 (35.7)
North	Tanda	352/272	245	148 (60.4)
	Meerut	332/321	282	56 (19.9)
	Delhi	1488/1209	1070	550 (51.4)
	Rohtak	501/269	246	86 (35.0)
	Ludhiana	479/477	384	136 (35.4)
	Jabalpur	79/79	65	13 (20.0)
	Bhopal	76/76	48	16 (33.3)
	Total	3307/2703	2340	1005 (42.9)
	Total	14315/11898	10207	4042 (39.6)

[*]*Data from hospital records;* [#] *data from validated data analysis.*

severe disease. Genotype analysis of a subset of rotavirus positive samples showed that G1 P [8] was the predominant strain (62.7%; 1579/ 2519). Clear history of rotavirus vaccination was available for only 3.3% (340/ 10206) of children.

DISCUSSION

The prevalence of rotaviral diarrhea among Indian children aged less than 5 years included in ENRSN (September 2012 to December 2014) was 39.6%. This is in conformity with the findings of the earlier round of NRSN (2005-2009) [5,6]. Inclusion of fewer hospital sites in the first phase of surveillance (2005-2009) limited the generalizability of the surveillance findings due to limited geographical representation.

The notable improvements in ENRSN included more involvement of government medical colleges / facilities which has led to capacity building in these centers for undertaking surveillance activities. All the project staff has been trained, and they are following identical protocols for enrolment, sample collection, clinical and laboratory procedures, and data management. The CRF has been modeled on the online data entry platform in the current round of surveillance. The CRF has been made simpler and more user friendly and data on rotavirus

WHAT IS ALREADY KNOWN?

- First phase of rotavirus surveillance in India (2005-09) documented the rotavirus disease burden at selective sites in the country.

WHAT THIS STUDY ADDS?

- Expanded National Rotavirus Surveillance Network validates the findings of the previous surveillance, highlights the high prevalence (39.6%) of rotavirus disease burden across the country, and documents baseline national level data at the point of rollout of rotavirus vaccine in the national immunization program.

vaccination history and dosage is being systematically collected. Through regular supervision and monitoring, focus is being given on quality management across the network.

The limitation associated with this hospital-based surveillance program was its inability to generate community-level disease burden data. Moreover, despite establishing a large network of 28 hospital sites across the country, some states are still outside the coverage of the network.

Only a small proportion of children attending various hospitals in the network had history of rotavirus vaccination. Commercial rotavirus vaccines are available in India since 2007-2008, and rotavirus vaccine is part of the optional vaccines recommended by the Indian Academy of Pediatrics. The high price of these vaccines drove them out of reach of the common man restricting their usage to a small proportion of children from affluent sections of Indian society. This may be one reason why a substantially large population of children continued to get infected and consequently developed severe diarrhea. It is anticipated that the launch of indigenous rotavirus vaccine in the national immunization program may result in substantial reduction in the morbidity and mortality associated with rotaviral gastroenteritis in the country, especially in areas with high rotavirus diarrhea burden.

Possible linkages between ENRSN and other surveillance networks in India like hospital-based sentinel surveillance of bacterial meningitis would result in better implementation and coordination of one or more surveillance programs in the same hospital setup. The network platform at various levels *viz*. district, state or regional levels, could cater to hypothesis generation for commissioning satellite studies for locally relevant issues. Besides surveillance for rotavirus, the ICMR supported ENRSN also offers appropriate infrastructure to conduct facility-based surveillance for other related pathogens and supplementary studies such as economic analysis and cost-effectiveness studies related to vaccine introduction.

In future, ENRSN will provide a unique opportunity for assessment of impact of vaccination on rotavirus disease in post-rotavirus vaccine introduction in India in a phased manner by estimating rotavirus disease burden, severity of disease, circulating genotypes as well as vaccine related severe adverse events like intussusception. A pilot retrospective case-record based survey for intussusception has been carried out involving several hospitals in Chennai (unpublished report), and efforts are on to conduct a larger multi-site survey and surveillance for intussusception using the ENRSN network platform. The surveillance is currently ongoing in 19 states/ UTs and newer hospital sites in states outside the coverage of the network will also be considered for inclusion to generate micro-level data on rotaviral disease burden in future.

Contributors: SMM: Network coordination, manuscript conceptualization and writing; SV and CPGK: contributed to network coordination, and manuscript writing; GK: Coordinated laboratory activities in the network and provided intellectual inputs for manuscript writing; MDG and RA: Coordination at ICMR level and provided intellectual inputs to for manuscript writing. All authors approved the final manuscript.

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ANNEXURE 1

Participating investigators of National Rotavirus Surveillance Network [2012 – 2016]

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