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ROTAVIRUS SYMPOSIUM

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A DECADE LONG STUDY ON GROUP A ROTAVIRUS (GARV) INFECTION AND GENOTYPE DIVERSITY AMONG CHILDREN WITH ACUTE GASTROENTERITIS IN KOLKATA, EASTERN INDIA (2012-2022)



Dr. Shanta Dutta
Director & Sc. G
ICMR-National Institute of Cholera Enteric Diseases
Kolkata, INDIA

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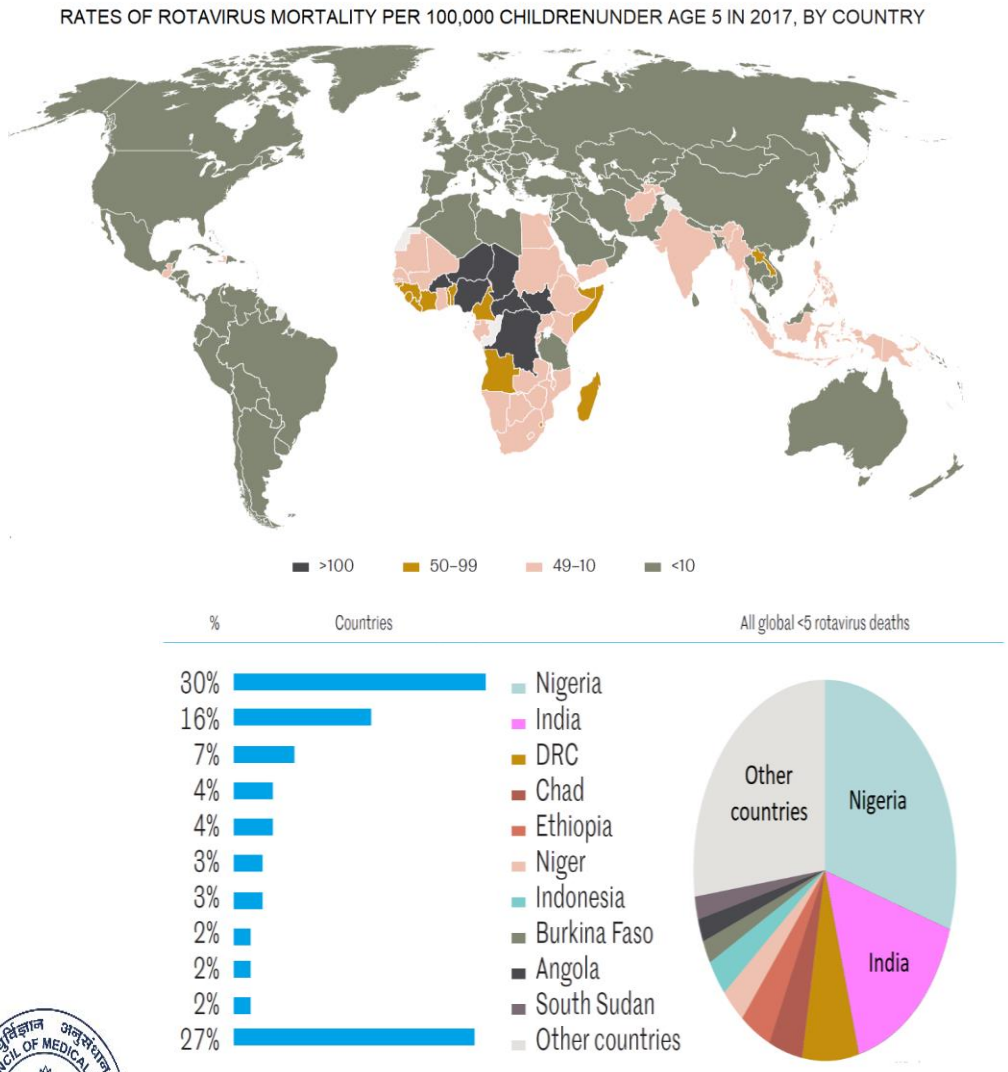
A brief Introduction of ICMR-NICED, Kolkata, India

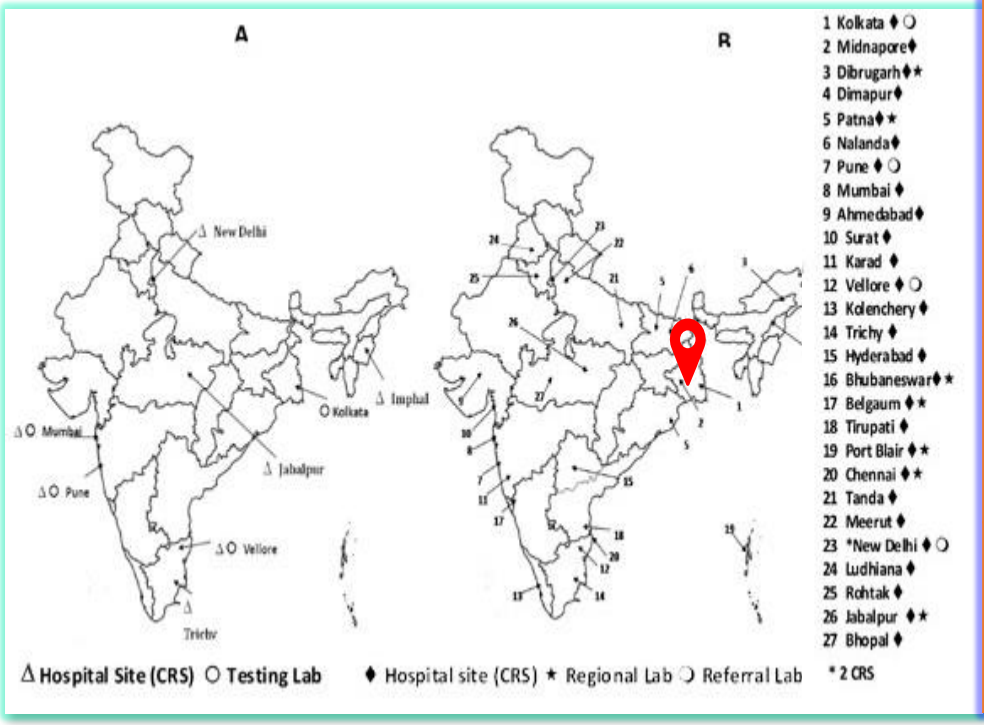
- ICMR-NICED started its journey in 1962 as a Cholera research center. It became a national institute for research on diarrhoeal diseases including cholera in 1979.
- In the year 1980, WHO recognized this Institute as "**WHO Collaborative Centre for Research and Training on Diarrheal Diseases"**
- The institute is involved in **both operational, applied and basic research** on pathogens (bacteria, viruses and parasites) causing enteric infections. More over researches on **HIV/AIDS, Influenza and Dengue** viruses are carried out.
- The institute has successfully conducted **efficacy and immunogenicity trials** for Cholera vaccine, Typhoid vaccine, Rotavirus vaccine and COVID 19 vaccines.
- In 2019, **National Antimicrobial Resistance Hub and National Repository of AMR Bacteria** were established in order to augment AMR research across the country and direct AMR related programs and Policies for mitigating the problem.
- The institute has a long history of collaborations with international agencies such as **WHO, John Hopkins University, International Vaccine Institute (IVI), CDC USA, NIID JAPAN, Okayama University Japan and JICA**

Rotavirus: a major causative agent of acute infantile diarrhea



- According to the 2017 Report of Rota Council, India accounted for 16% of Group A Rotavirus (GARV) related childhood mortality.
- To bring down rotavirus mortality rates in India, phased roll out of Rotavirus vaccine in UIP was initiated in **2016.**
- Two Made in India vaccines licensed for phase roll out in Indian states were
  - **RotaVAC** (strain: G9P[11]; Manufacturer: Bharat Biotech, India) and
  - **Rota SIIL** (Pentavalent vaccine; strains G1,G2,G3,G4, G9 and P[8]); Manufacturer: Serum Institute of India)
- **By end on 2019, RV vaccine was introduced in UIP in all states**

The National Rotavirus Surveillance Network



Sites of surveillance (NRSN)	Number of samples screened	Number of Rotavirus positives	Percentage positivity
East India (West Bengal, Assam, Nagaland, Bihar, Orissa)	5846	2328	**39.80%**
West India (Maharashtra, Gujarat)	3710	1272	**34.30%**
North India (Punjab, Himachal Pradesh, Uttar Pradesh, New Delhi, Haryana, Madhya Pradesh)	4471	1684	**37.70%**
South India (Tamil Nadu, Andaman and Nicobar, Andhra Pradesh, Telengana, Kerala, Karnataka)	7394	2499	**33.80%**

- **Conducted in 28 sites in 19 states/Union territories of India (2012-2016).**

- **Eastern region had highest proportion of rotavirus-associated diarrhea (39.8%).**
- **Site wise, rotavirus positivity was the highest in Bhubaneswar in Odisha state of India (54.9%), followed by Midnapore (West Bengal; 53.5%).**

Provided necessary baseline data for introduction of RV vaccines in 2016

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The Surveillance Sites

Brief methodology

METHODOLOGY

- Fecal sample collection from children (<5 yr) with diarrhea
- Preparation of viral suspension (VS)
- Screening for GARV positivity by GARV-specific ELISA
- Nucleic acid isolation (RNA for GARV)
- Reverse transcription of RV RNA
- Amplification of GARV outer capsid genes for genotyping: VP4 & VP7 specific primers
- Sequencing of GARV VP4 and VP7 gene by Sanger sequencing method
- Phylogenetic analyses using software like BLAST, LALIGN, CLUSTALW, TRANSEQ & MEGA 11;
  .

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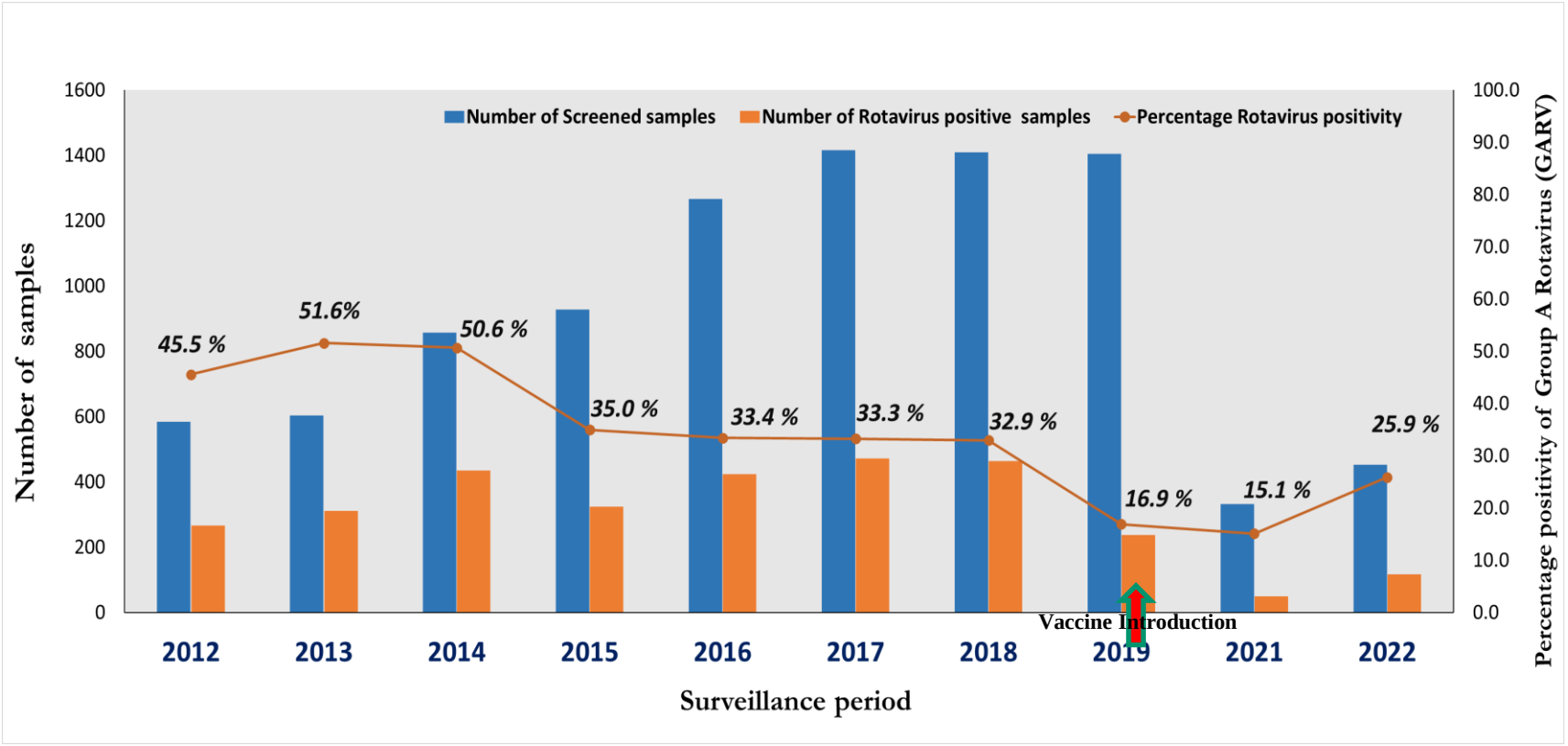
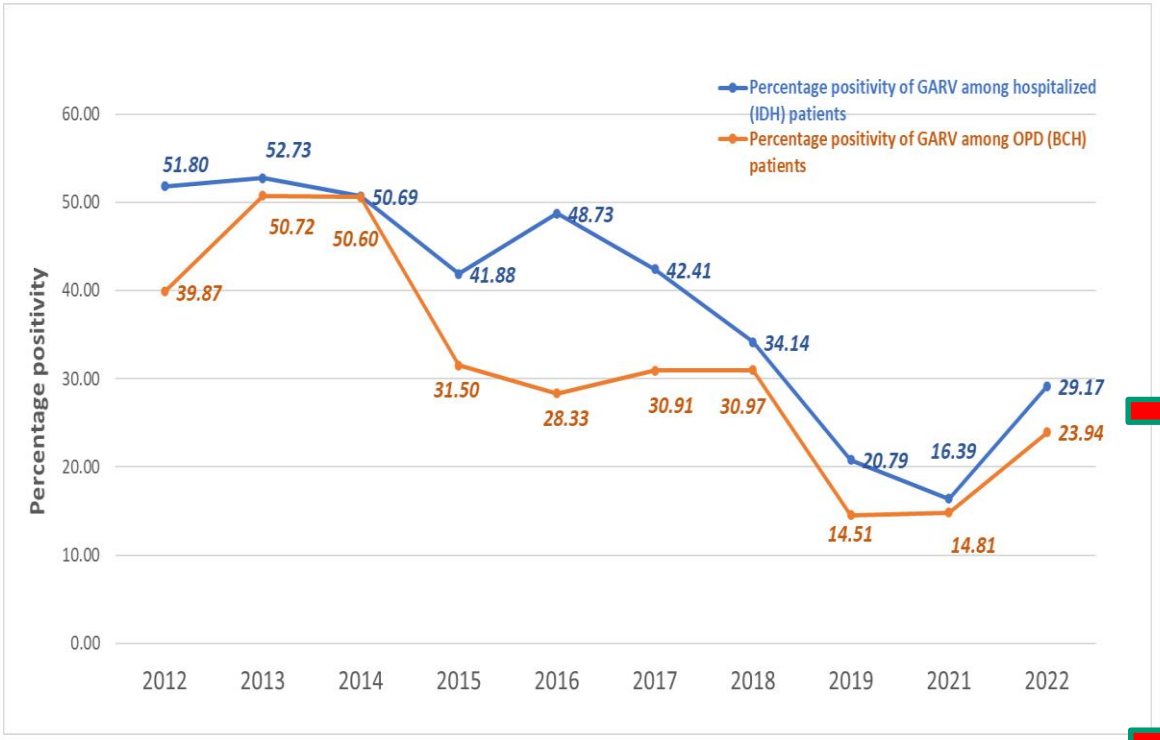
Proportion of GARV among children (≤5 years) with acute gastroenteritis in West Bengal over a decade (2012-2022)



- GARV positivity rates among children varied between **45-50% during 2012-2014** reduced to **32-35% during 2015-2018**.
- **A decline in positivity rates of GARV was observed during 2019-2022,** with concomitant reduction in diarrheal cases.
- No surveillance could be conducted during 2020 due to the ongoing COVID-19 pandemic.

Comparison of GARV positivity rates among hospitalized children (≤5 years) vs out-patient visits



	GARV-Positives (n = 1352/2588)	GARV-Negatives (n = 1236/2588)
(a) Frequency of stool passage/24 h		
≤5 (n = 31)	15	16
≥6 (n = 2557)	1337	1220
		χ^2 = 0.186, p-value = 0.665
(b) Degree of dehydration		
None (n = 879)	505	374
Some (n = 1592)	802	790
Severe (n = 117)	45	72
		χ^2 = 20.686, p-value = 0.000032
(c) Duration of stay at the hospitals		
<3 days (n = 1177)	568	609
≥3 days (n = 1411)	784	627
		χ^2 = 13.725, p-value = 0.000212
(d) Fever		
≤37 °C (n = 1621)	843	778
37.1 °C-38.9 °C (n = 947)	495	452
≥39 °C (n = 20)	14	6
		χ^2 = 2.564, p-value = 0.277389
(e) Frequency of vomiting/24 h		
0 (n = 1060)	559	501
1 (n = 46)	22	24
2–4 (n = 837)	406	431
≥5 (n = 645)	365	280
		χ^2 = 10.029, p-value = 0.018316

- **GARV positivity rates among hospitalised children were higher**
- GARV infections correlated with **increased frequency of vomiting** and **greater duration of stay at the hospital for treatment** compared to GARV negative gastroenteritis cases.

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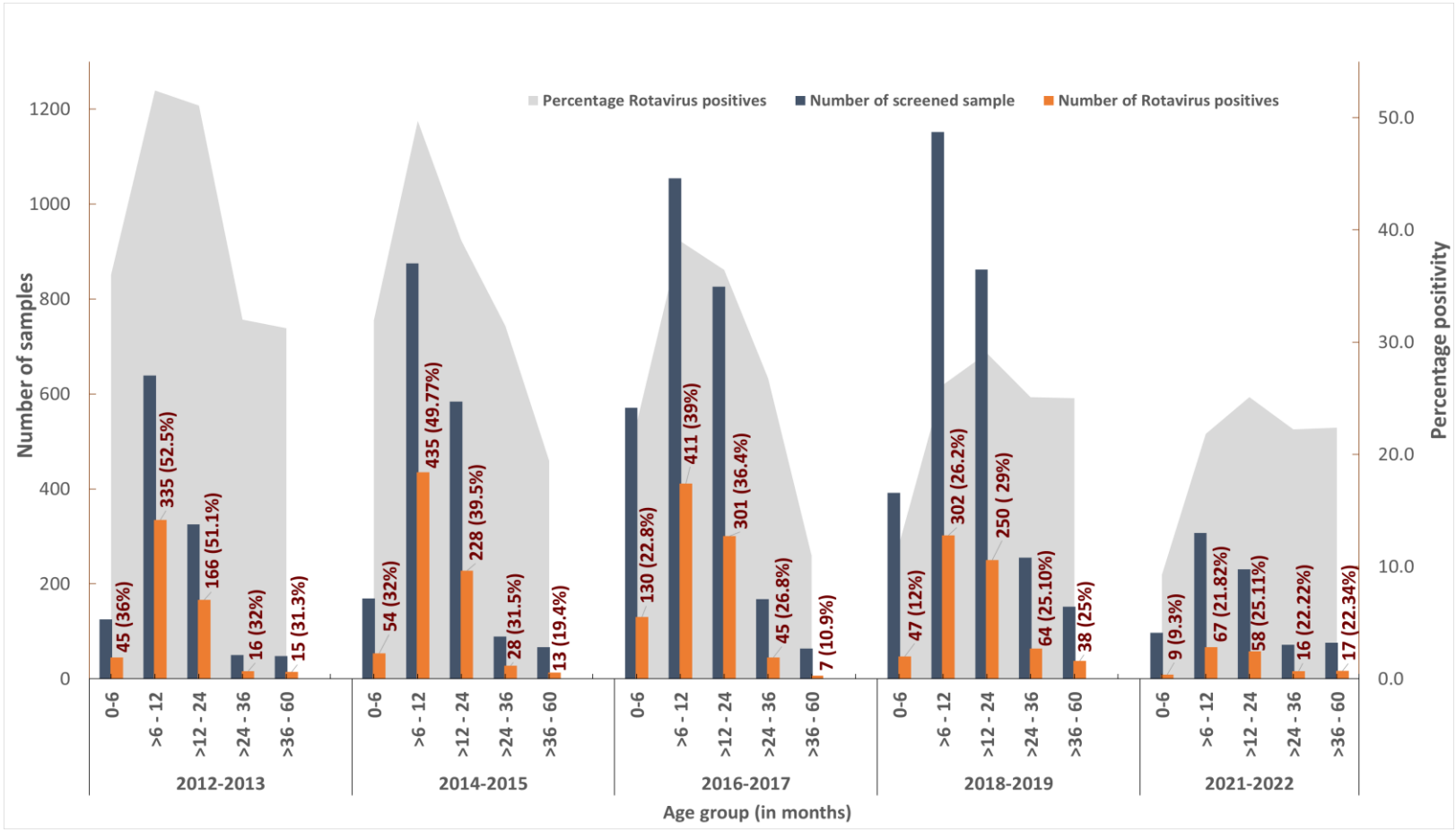
Age-wise distribution of GARV positivity



- **GARV positivity was highest among children aged between 6-24 months.**
- As Rotavirus positivity rates started to decline after 2018, increased positivity has been observed in age groups >24 months.

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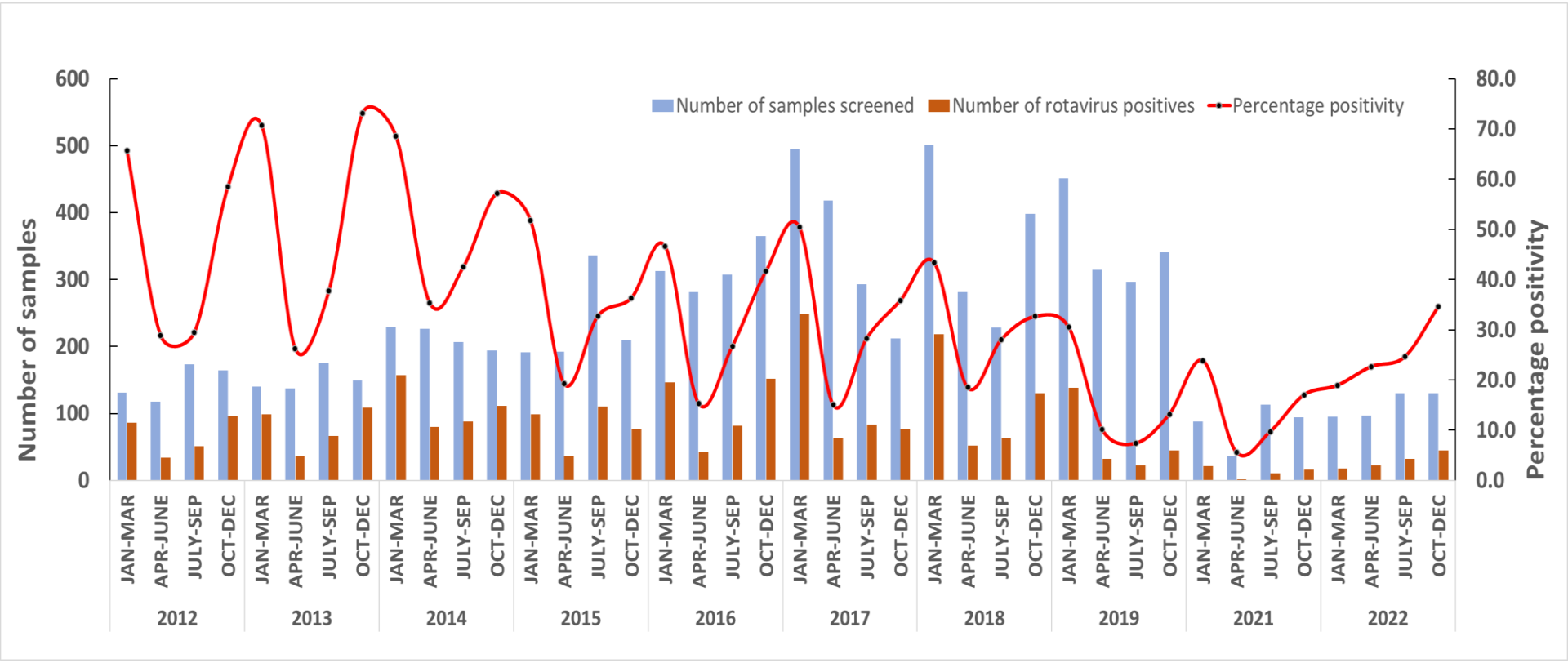
Seasonal distribution of GARV infections in West Bengal



- **GARV infection was observed through out the year but it peaked during the cooler months (October- Feb) with lower precipitation rates.**
- Rates of GARV infections were significantly low during warm and wet months (April-September)

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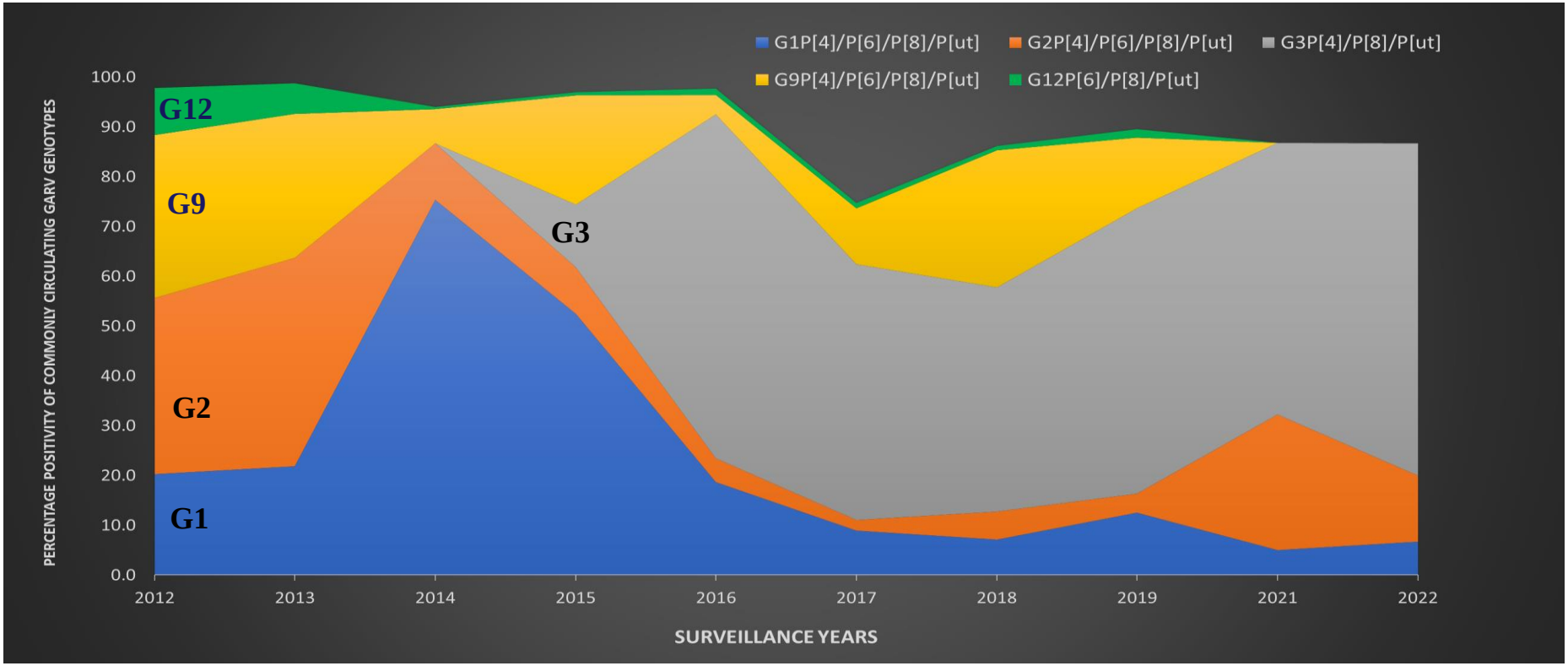
Dynamicity in circulating GARV genotypes in West Bengal



- **G1, G2 and G9 genotype** of GARV circulated during **2012-2013**
- **G1 genotype** predominated during **2014-2016.**
- **G3 genotype** was first detected in Kolkata, **in 2014 and soon outcompeted G1,**
- becoming the **predominantly circulating strain** since **2016**.
- Rotavirus vaccine was introduced in **UIP of West Bengal on 21st August, 2019**.
- **G3** has remained the predominantly **circulating type as of 2022**
- **G9 genotype** circulating moderately **till 2019**
- was **not detected** in any of the rotavirus positive samples **between 2021-2022.**

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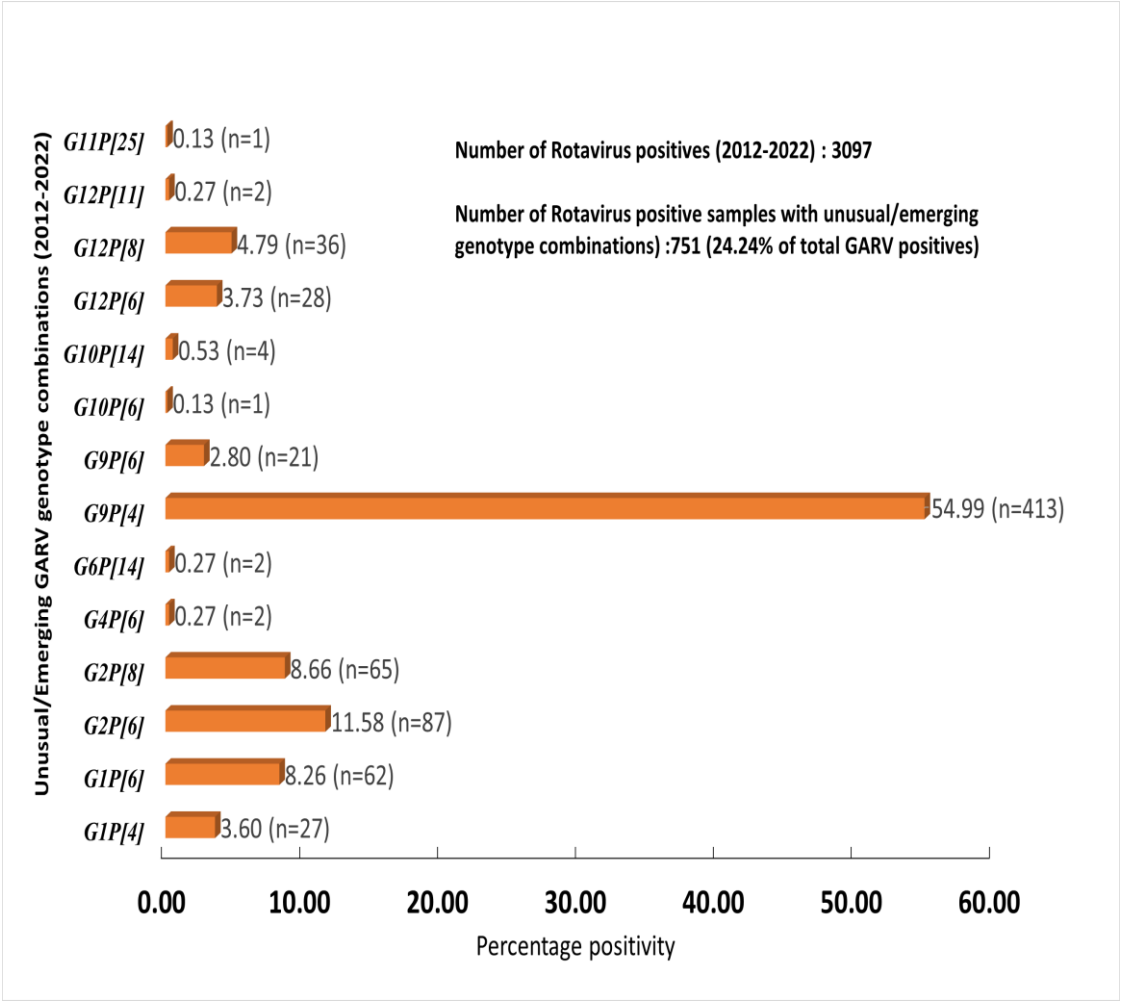
Circulation of unusual genotype combinations and animal-human re-assorted strains

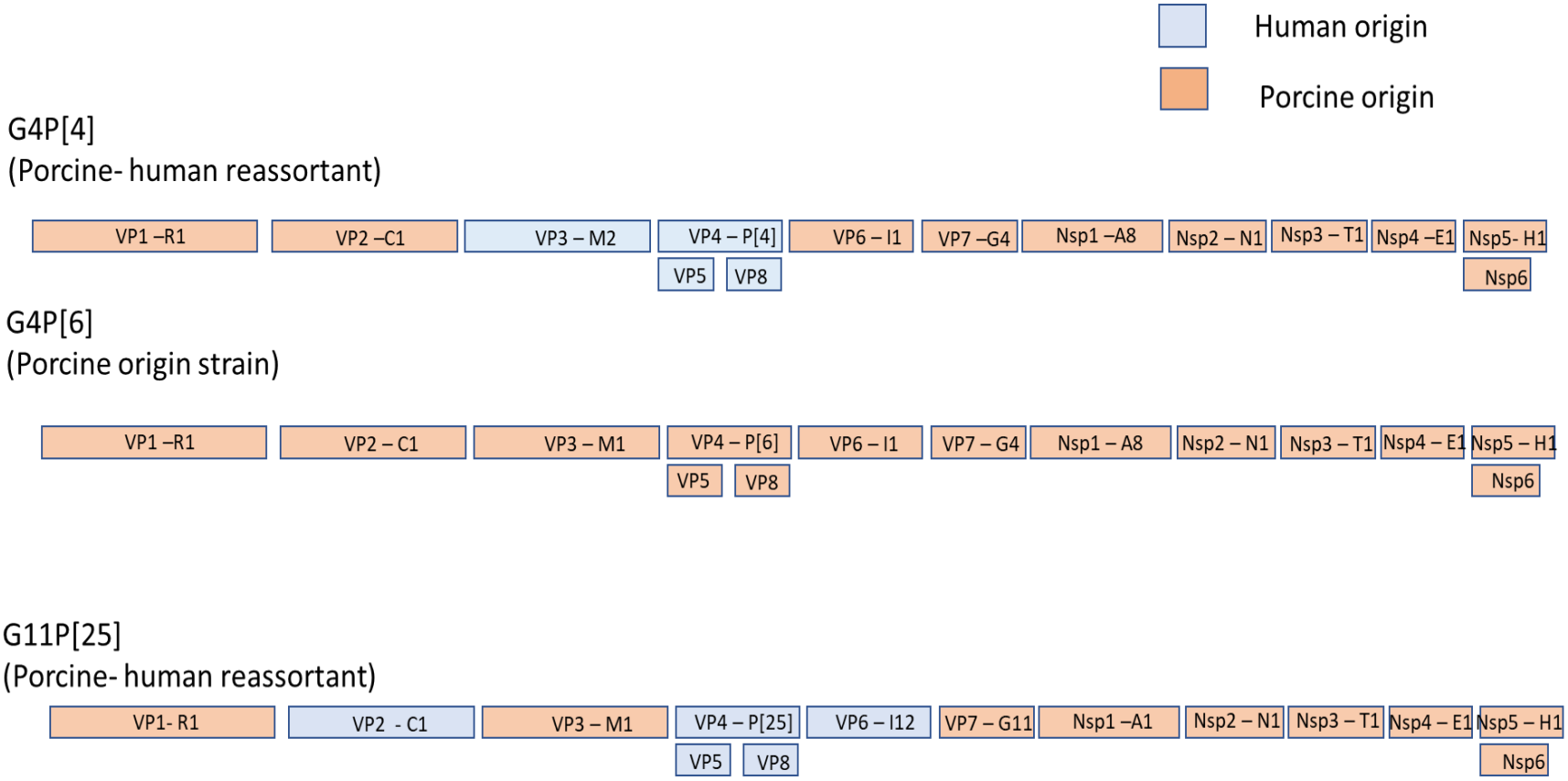
- Out of all the GARV 'GP' genotypic combinations obtained during 2012-2022 in West Bengal, India, 1/4th (24.24% of total GARV positives) comprised of **unusual combinations of circulating genotypes like G1P[4], G1P[6], G2P[6], G2P[8], G9P[4] etc.**

- **A few strains like G6P[14], G10P[14], G11P[25] of zoonotic origin were** identified

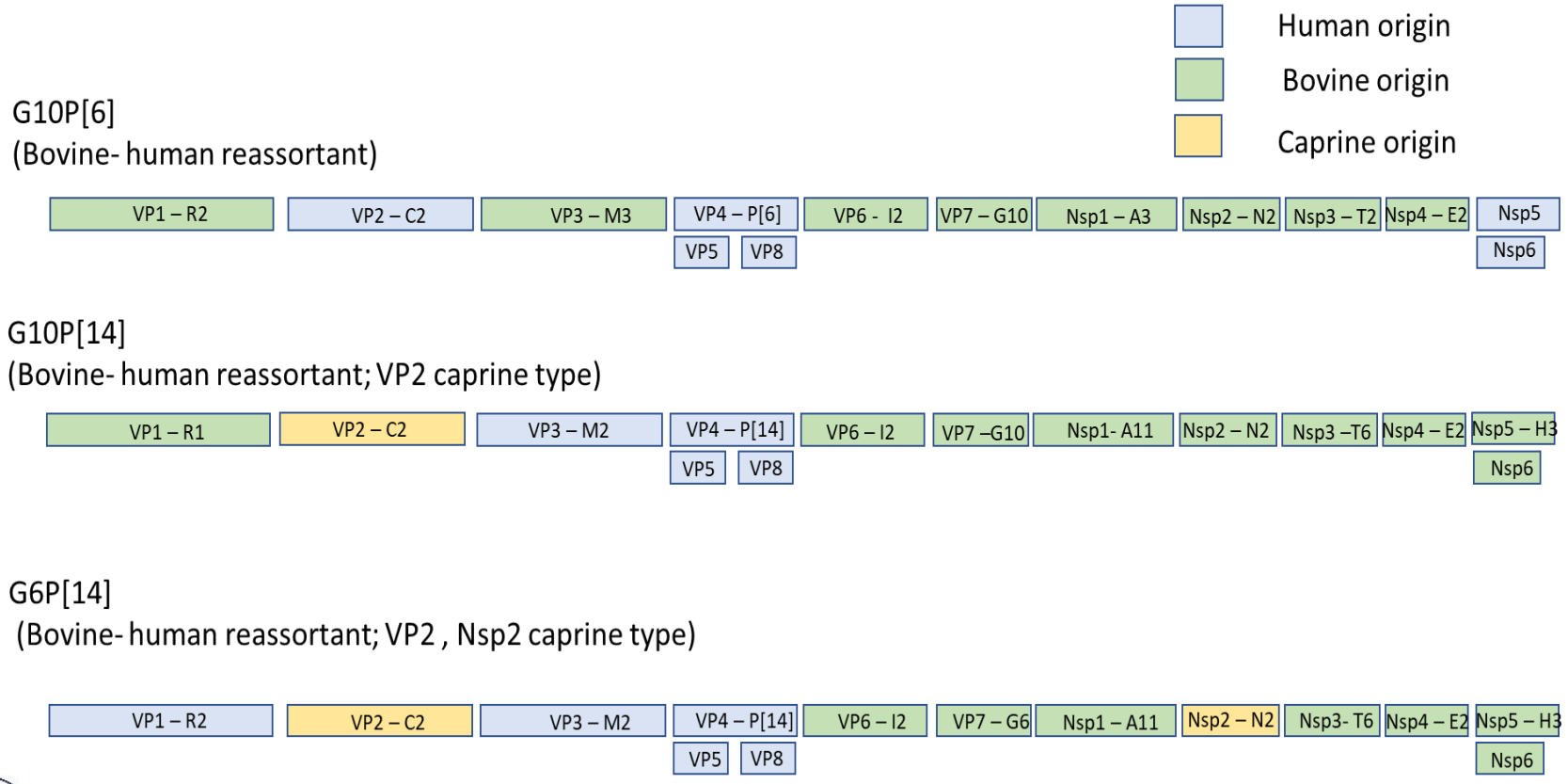
- During the NRSN study period of 2012-2016, **greater diversity in** circulating **GARV genotypes** was noted among strains from **urban areas** compared to strains from rural areas



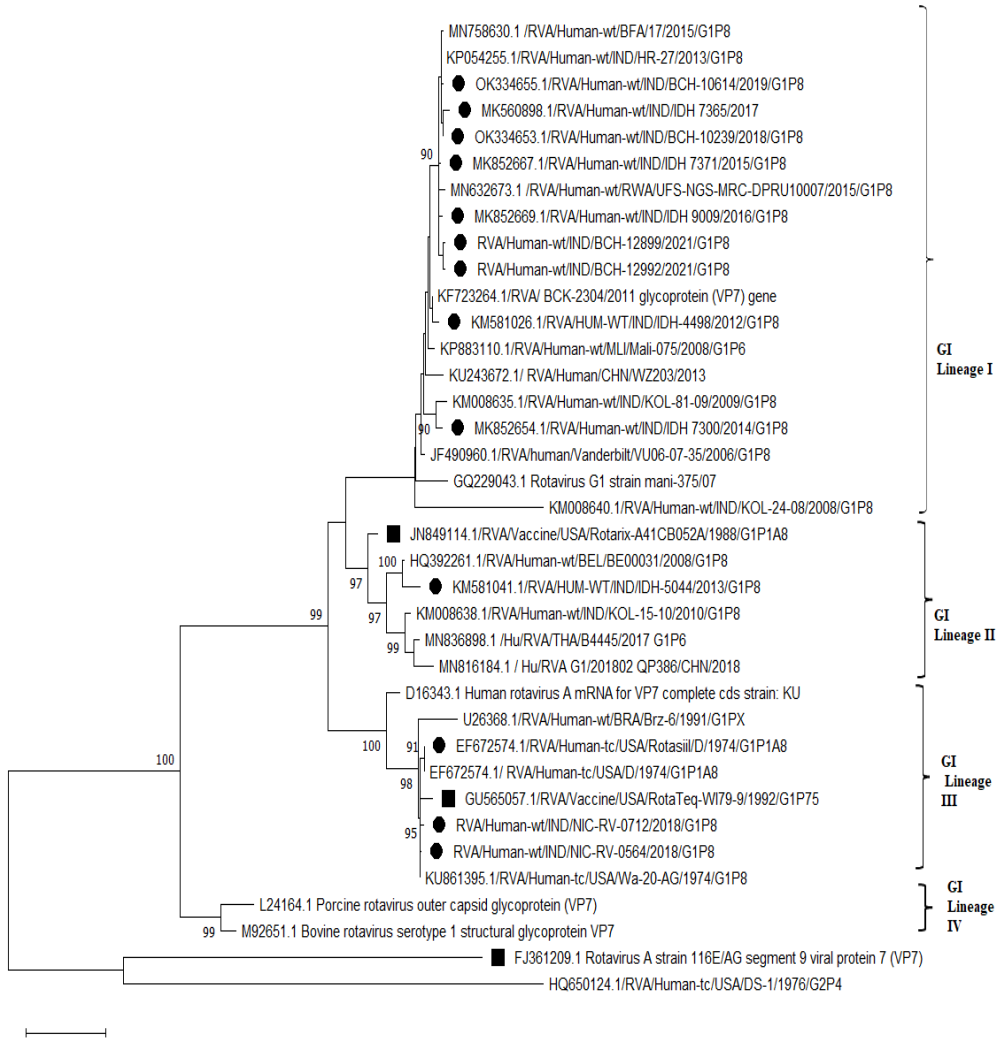
Genetic backbone of some animal-human re-assortant strains circulating in Kolkata, India during 2012-2022



Genetic backbone of some animal-human re-assortant strains circulating in Kolkata, India during 2012-2022 (contd.)



Phylogenetic analysis of G1 genotype strains (2012-2022)



- Most of the **G1 strains** circulating during **2012-2022** in Kolkata **belonged to Lineage I.** These strains bore **90-92% similarity with vaccine strains**.

- A few **G1 strains** of GARV circulating in Kolkata **were found to cluster in GI Lineage II along with the Rotarix** vaccine G1 strain.

- Only **two G1 strains circulating in 2018** had high nucleotide sequence homology to the **G1 strain in RotaTeq and RotaSIIL** (vaccine strains) and clustered together under **GI Lineage III.**

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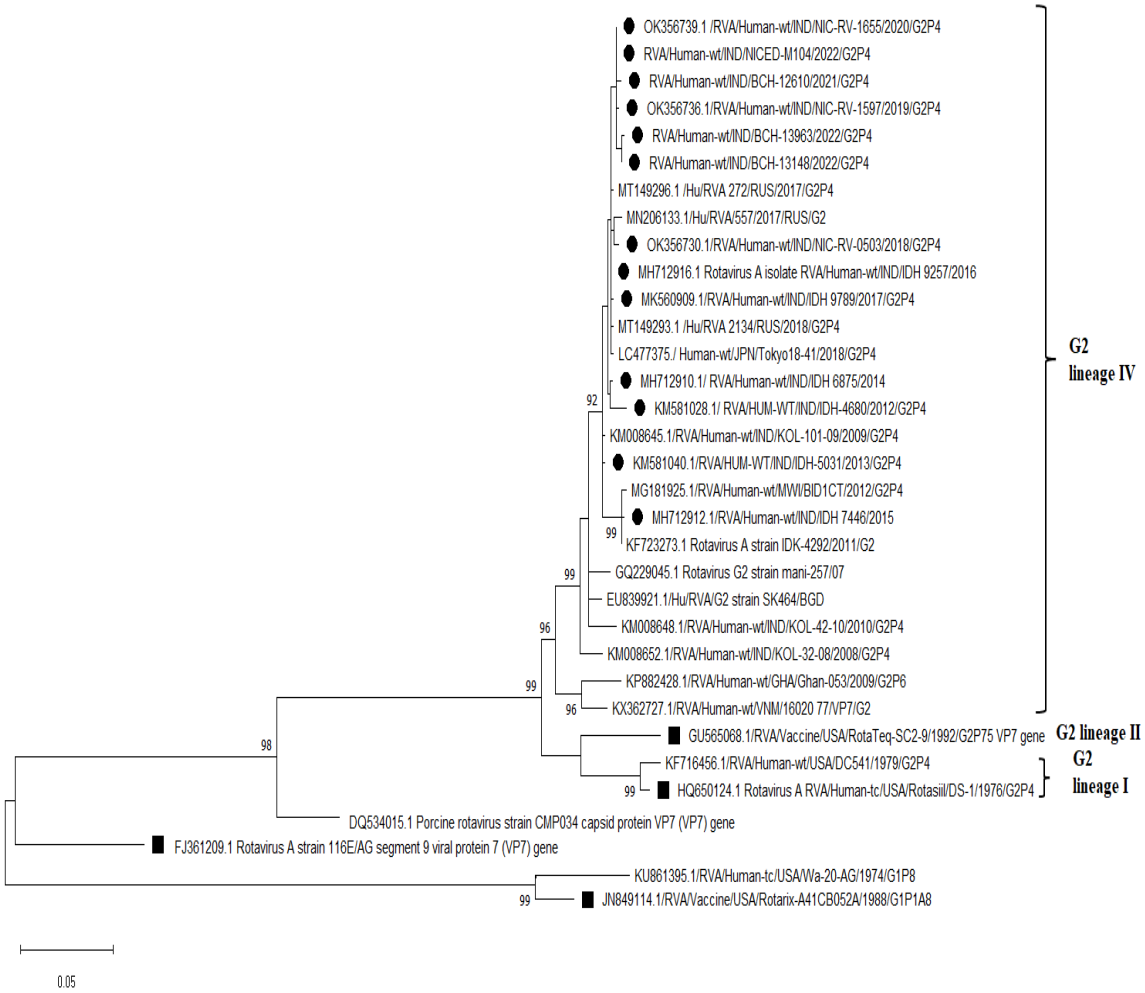
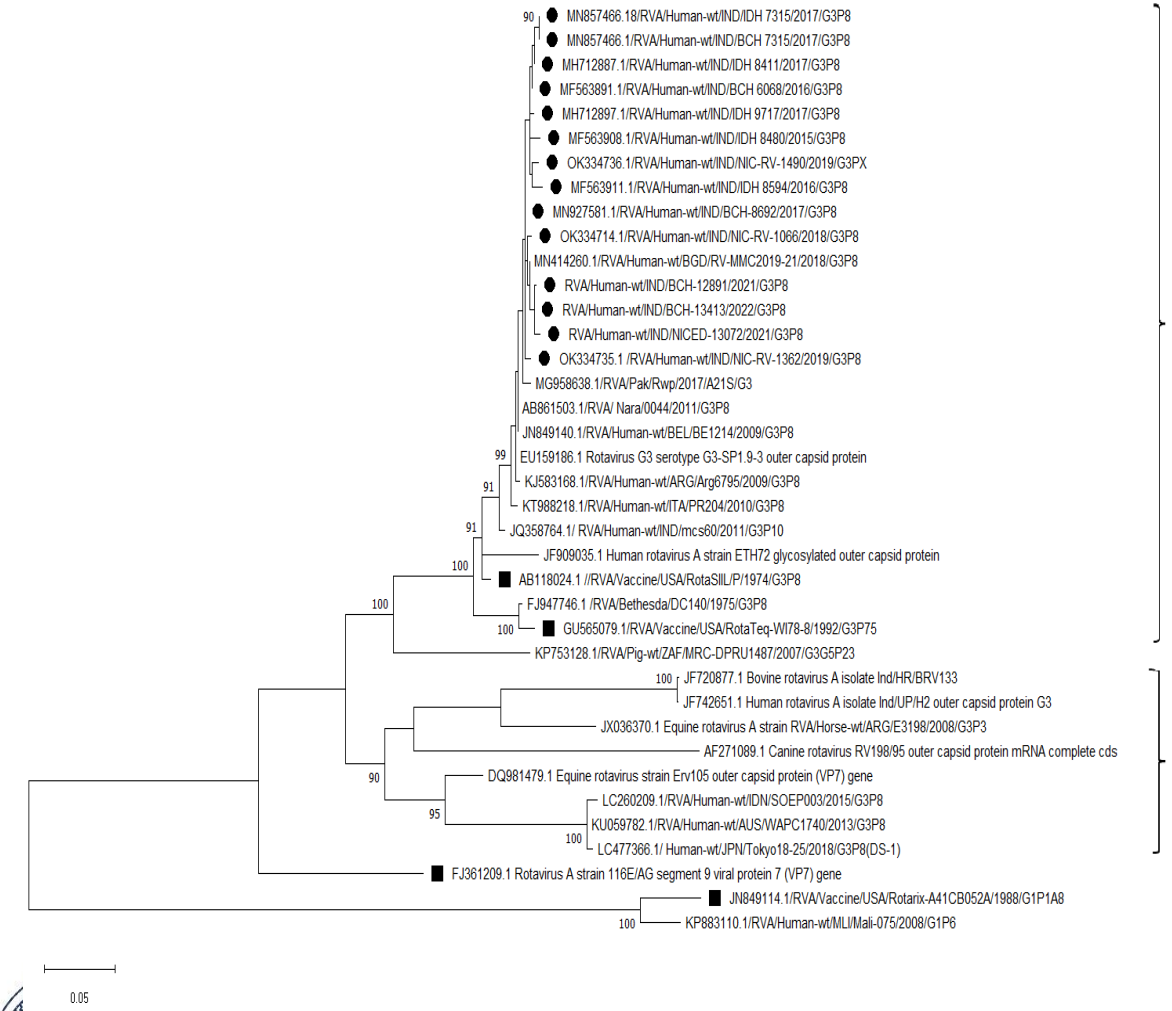
Phylogenetic analysis of G2 genotype strains (2012-2022)



- **G2 strains** of GARV circulating in Kolkata during **2012-2022** belonged exclusively **to G2 lineage IV**.

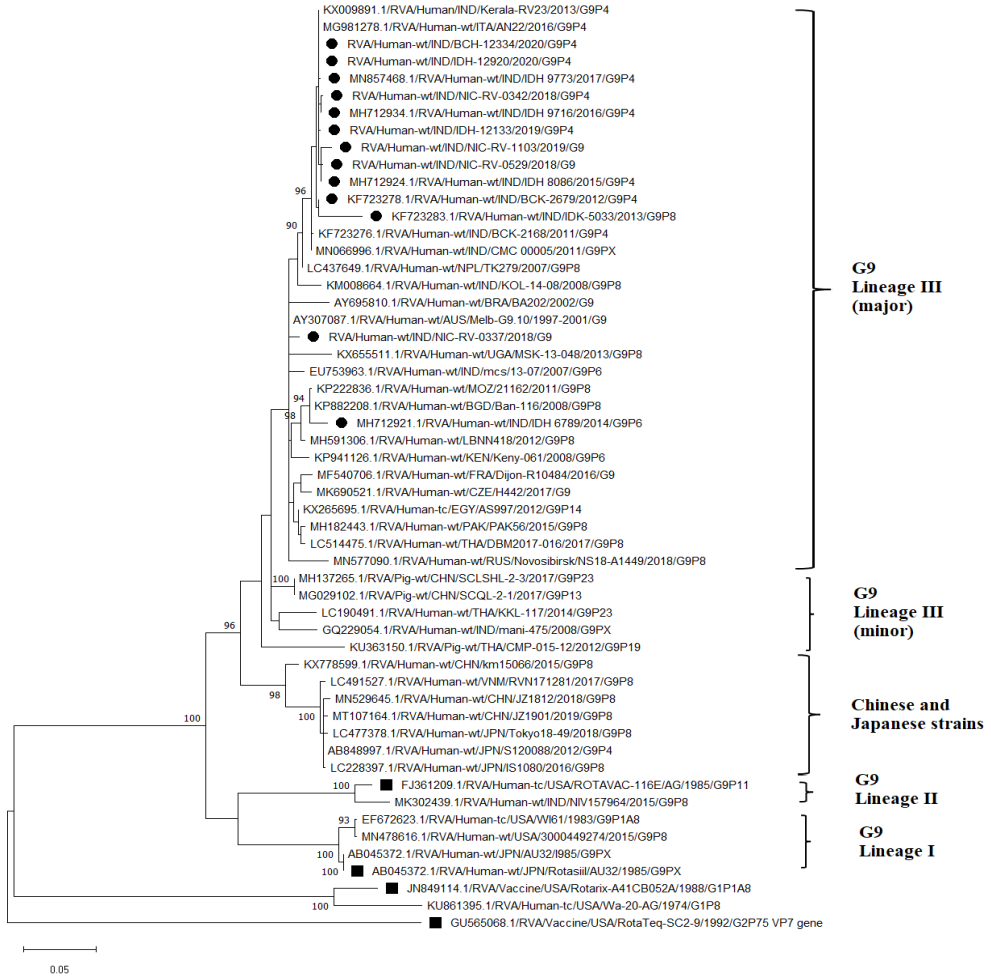
- These strains showed **90% nucleotide homology with the G2** in backbone of multi-valent **vaccines like RotaTeq and RotaSIIL**

Phylogenetic analysis of G3 genotype strains (2012-2022)



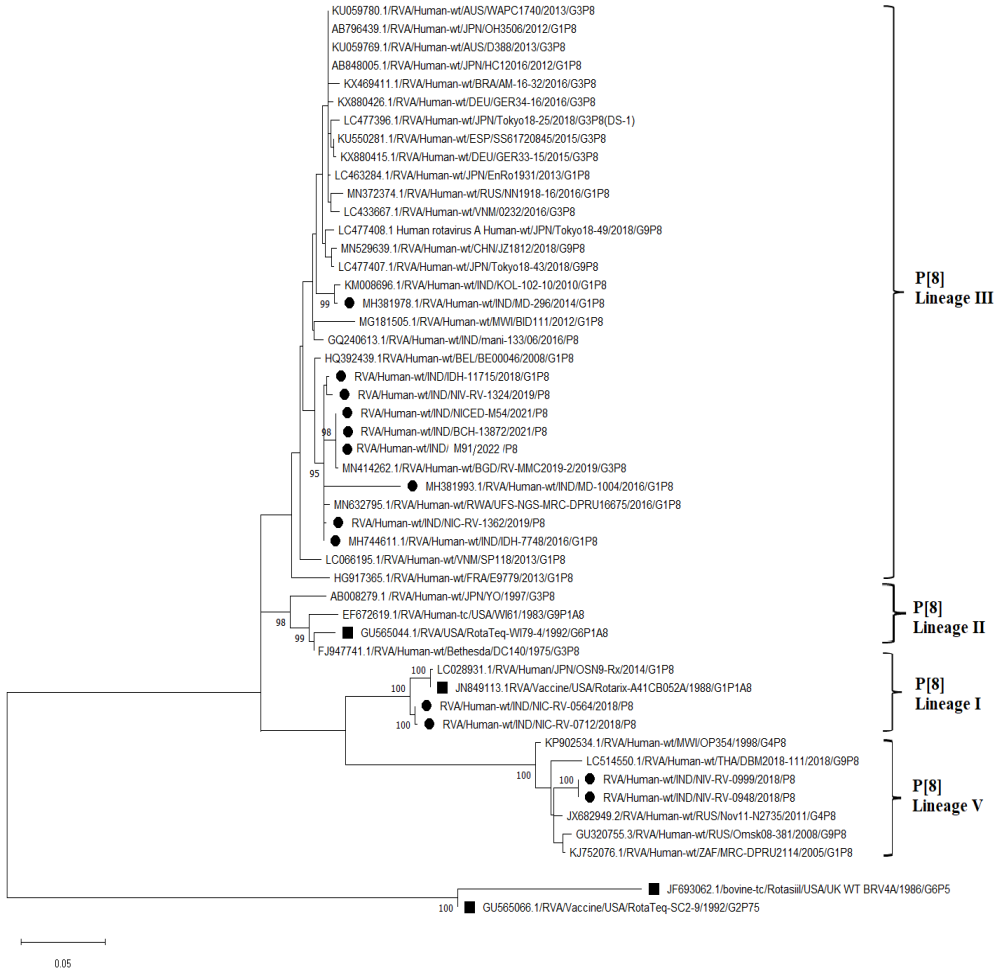
- **G3 strains** first detected in Kolkata in **2014,** has remained **the dominantly circulating type till 2022 .**
- All the **endemic G3 strains of 2015-2022** clustered in **G3b lineage.**
- **G3** in backbone of vaccines **like RotaTeq and RotaSIIL** clustered with the **endemic G3 strains**
- **None of the G3 strains** obtained were of **equine G3 type** which were frequently identified in countries like **Japan, Indonesia and Australia** during similar time period.

Phylogenetic analysis of G9 genotype strains (2012-2022)



- **G9 strains** circulating in Kolkata between **2012-2019** clustered together **in G9 major Lineage II strains,** along with G9 **strains circulating in Nepal, Uganda, Bangladesh**.
- **The endemic G9 strains** had **87-88%** nucleotide sequence **similarity with G9 strains in vaccine RotaVac and RotaSIIL.**

Phylogenetic analysis of P[8] strains (2012-2022)



- **Majority of P8 strains circulating during 2012-2022** in Kolkata clustered **in P[8] lineage III** and were genetically distant **to Rotateq (GU565044) and Rotarix vaccine strains.**

- **Two G1 P[8] strains from 2018** clustered in **Lineage I** along with **P1A8 strain of Rotarix vaccine**

- **Two other P[8] strains from 2018** were found to cluster with **the lineage V OP354 P[8] strains** which display high **degree of heterogeneity at antigenic epitopes with the vaccine strains.**

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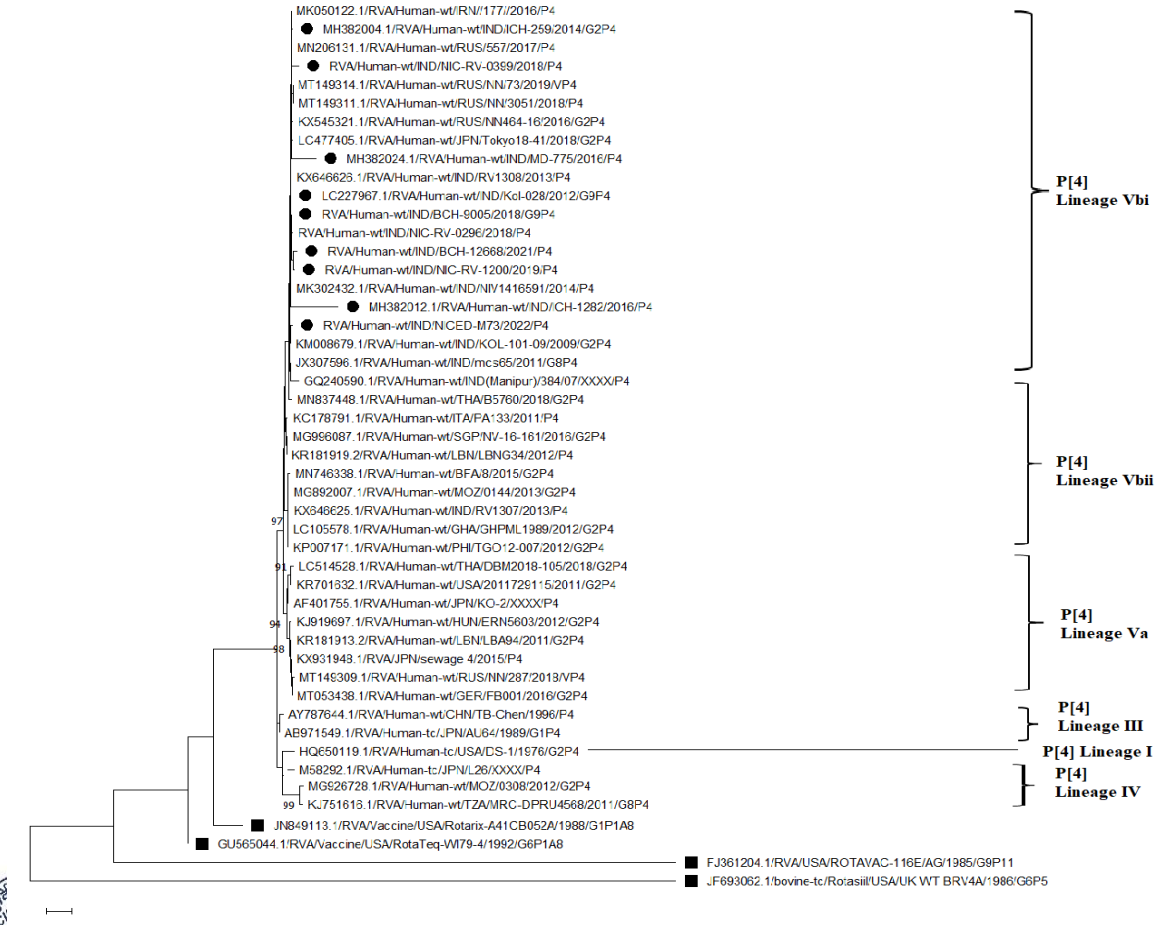
Phylogenetic analysis of P[4] strains (2012-2022)



- **All of the P[4] strains circulating between 2012-2022 clustered in Lineage Vbi** along with P4 strains detected in Russia during 2016-2019.
- **The representative P[4] strains revealed 85–86% DNA sequence similarity with** the P-type backbone of **vaccine strains, Rotarix (JN849113) and RotaTeq (GU565044).**

Variations in amino acids at antigenic epitopes of VP7 protein of circulating genotypes (2012-2022) with respect to the licensed vaccine strains

G1 :

	7-1a														7-1b						7-2								
	87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
Rotarix G1	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
RotaTeq G1	T	T	N	G	D	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	S	L	S	M	N	G
RotaVac G9	I	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	N	S	T	L	S	E	N	G
RotaSIIL G1	T	T	N	G	G	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	S	L	S	M	N	G
IDK-4226/2011/G1; MK829338/2015/G1; OK334659/IDH-11306/2018/G1	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
BCK-2304/2011/G1; MH852639/2015/G1; OK245385/NIC-RV-1588/2020/G1	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
MH381853/2015/G1; OK245379/NIC-RV-1247/2019/G1; BCH 12899/2021/G1	T	N	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
OK245369/NIC-RV-564/2018/G1	T	T	N	G	D	W	K	D	Q	N	V	V	D	K	Q	N	V	D	N	T	K	D	Q	S	L	S	M	N	G

G3 :

	7-1a														7-1b						7-2								
	87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
Rotarix G1	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
RotaVac G9	I	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	N	S	T	L	S	E	N	G
RotaTeq G3	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	A	N	K	D	K	D	A	T	L	S	E	A	G
RotaSIIL G3	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
G3P[8] IDH 2016; OK334725/NIC-RV-1272/2019/G3	I	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
G3P[8]/BCH 2015; OK334728/NIC-RV-1297/2019/G3; BCH-18413/2022/G3	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G

Summary

- **The decrease in the Rotavirus positivity rates** in the state is evident after **the roll out of GARV vaccine** in Universal Immunization Program in West Bengal in 2019
- Among hospitalized children, rotavirus positivity correlated with **increased in vomiting episodes and longer duration of hospital stay** compared to the other causes of gastro-enteritis.
- During **2012-2017 maximum i**nfection rates **were observed in 6-24 months** age group. **After 2018** a shift has been observed towards **age group of > 24 months**. Further follow up studies in the post-vaccine era may confirm **relevance of this shift in age group**
- **During 2012-2022**, the predominantly **circulating GARV genotypes were G1P[8], G2P[4] and G3P[8]. G9P[4]** genotype circulated during **2012-2018** but has **not been observed after 2019.**
- The RV vaccine was **introduced in August 2019** in the study area, but the **Pandemic in 2020** has disrupted the equilibrium. Over all diarrhea cases reporting to hospitals were very low due to lock down and treatment directed to only COVID-19 positive cases in hospitals.

Thus **continuous surveillance during post-RV vaccination** era may further confirm the **impact of vaccination** on epidemiology and hospitalization of RV diarrhea . Continuous **genotyping of circulating GARV strains** is also crucial to detect **shift in genotypes and emergence of re-assortant or vaccine-derived strains.**

ICMR-NICED is equipped with high-end technological facilities for virology research

Acknowledgements

- I would like to acknowledge the organizing committee for providing the Travel grant to the Scientists and students from ICMR-NICED, Kolkata, India

- Funding support for research from Indian Council of Medical Research (ICMR) and Okayama University, Japan is gratefully acknowledged

- The surveillance is a team work of

  -Clinicians from partner hospitals like ID & BG hospital, BC Roy Post graduate Institute of Pediatric Sciences, Midnapur Medical College),

  -Clinical division at NICED

  -Enteric virus Lab of ICMR-NICED with Dr Mamta Chawla Sarkar as the Chief Scientist.

ICMR-NICED enteric virus lab members

Thank You

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