

FOURTEENTH INTERNATIONAL
ROTAVIRUS SYMPOSIUM

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GENOTYPIC SURVEILLANCE AND EPIDEMIOLOGIC TRENDS OF ROTAVIRUS INFECTION AMONG CHILDREN WITH GASTROENTERITIS IN BANGLADESH, 2014-2021

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Background

- Rotavirus has been reported as the main pathogen of pediatric acute gastroenteritis.
- Rotavirus is associated with half of total children diarrheal episodes worldwide (Kotloff *et al.*, 2017).
- The incidence of rotavirus is estimated to be 10,000 cases per 100,000 children aged <5 years in Bangladesh.
- Rotavirus associated mortality is estimated to be 2000 to 3000 per year in Bangladesh.

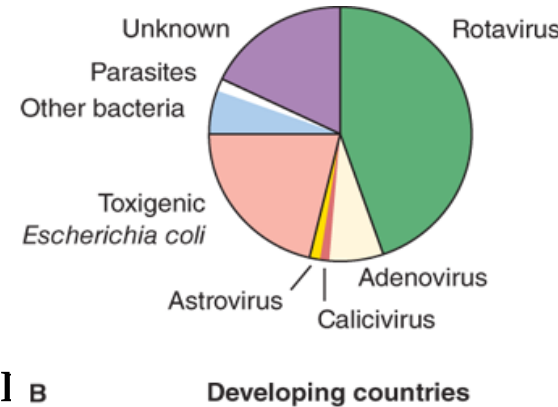


Fig 1. Rotavirus contributes to the majority of the cases of AGE



Source: <https://www.globalgiving.org>



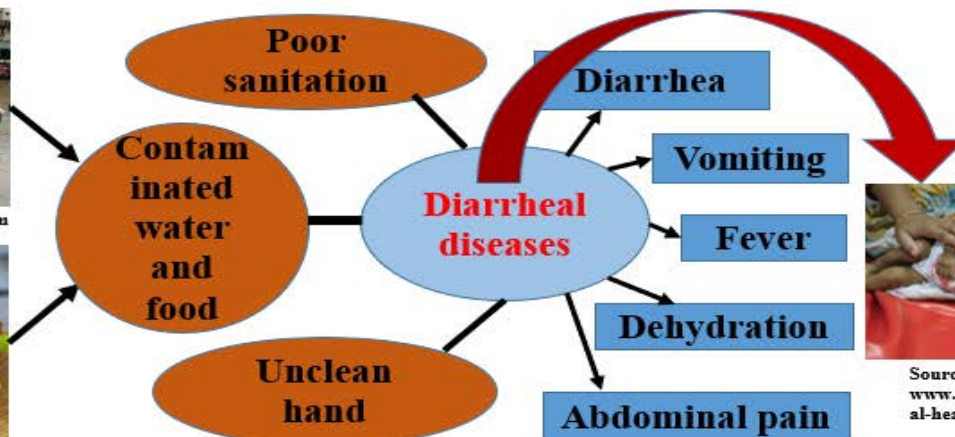
Source: <https://labonnonews.com>



Source: <https://ejatlas.org>



Source: <https://www.independent.co.uk>



Source: www.telegraph.co.uk/global-health

Materials and Methods

- A total of 838 fecal specimens were collected during Jan 2014 to Dec 2021 from four regions in Bangladesh.
- A one-step RT-PCR method was performed for the detection of rotavirus.
- Molecular sequencing was performed for the positive samples.
- Immunochromatography (IP-Noro/Rota, Japan) kit was used for rapid detection of rotavirus.

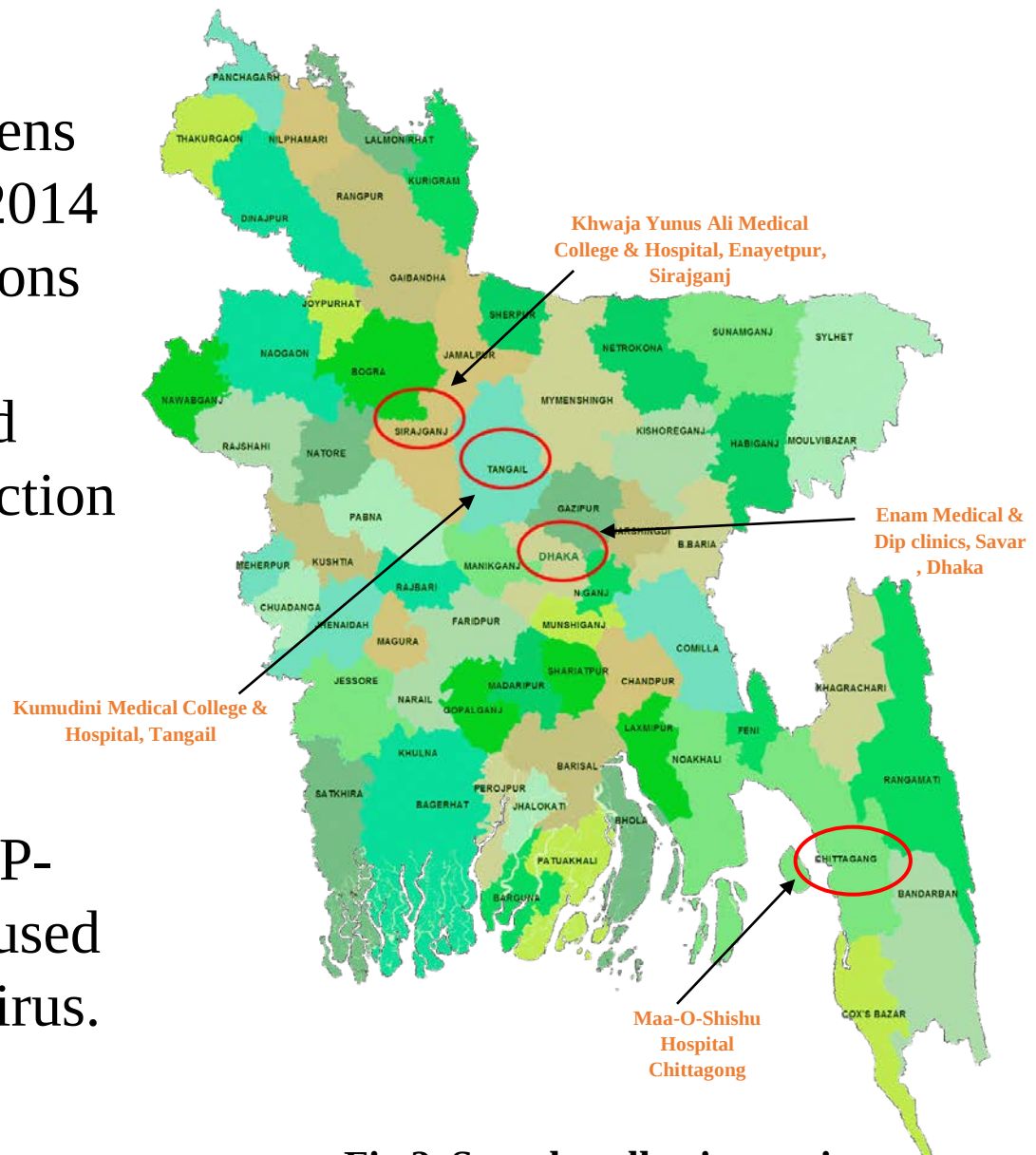
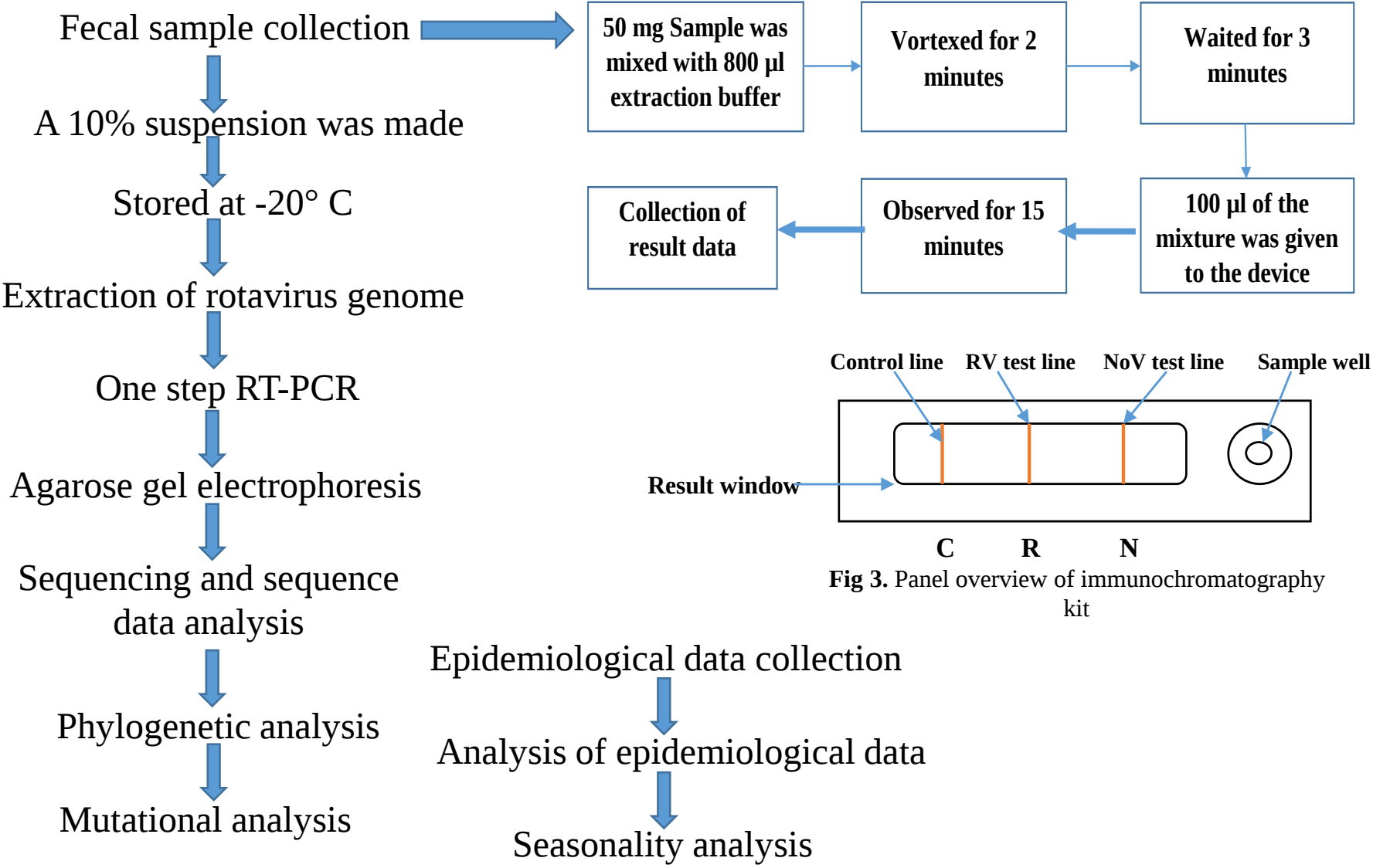


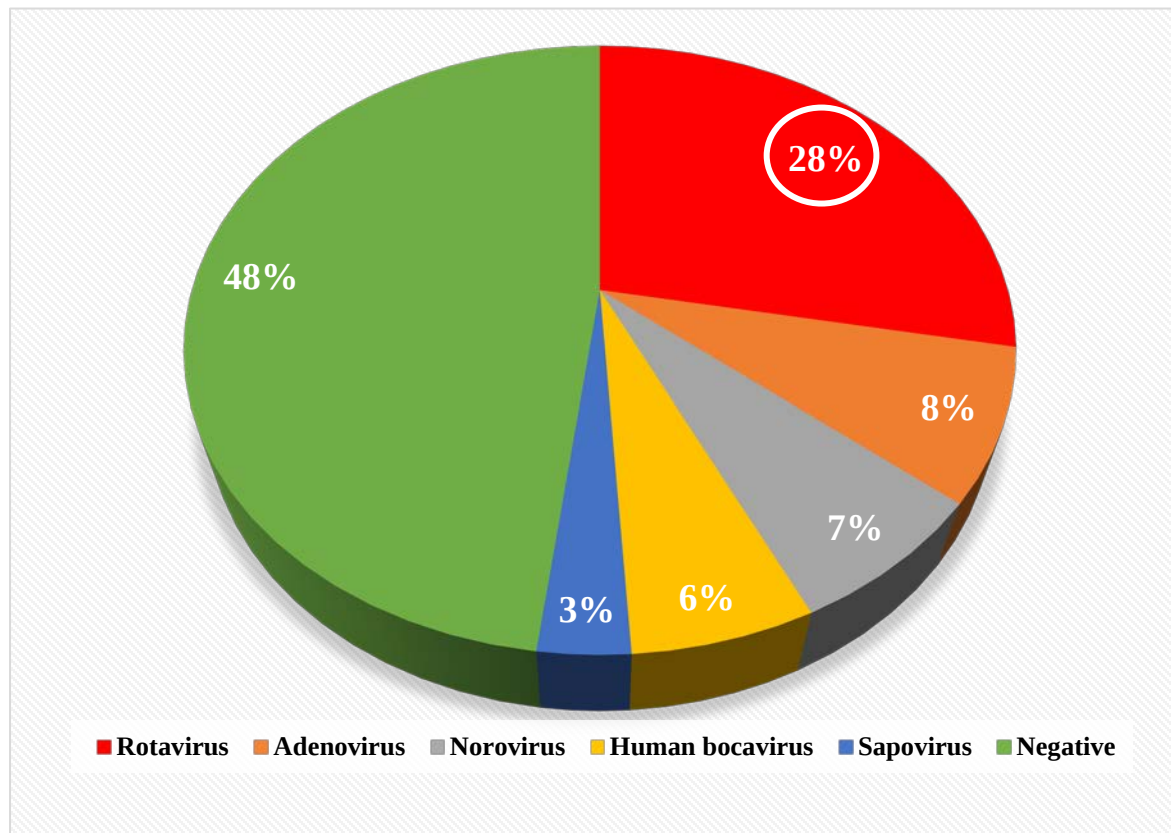
Fig 2. Sample collection regions

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Findings

- Rotavirus was detected in 231 out of 838 specimens (28%).
- Among other pathogens, norovirus was detected in 8% samples followed by adenovirus (7%), human bocavirus (6%) and sapovirus (3%), respectively.



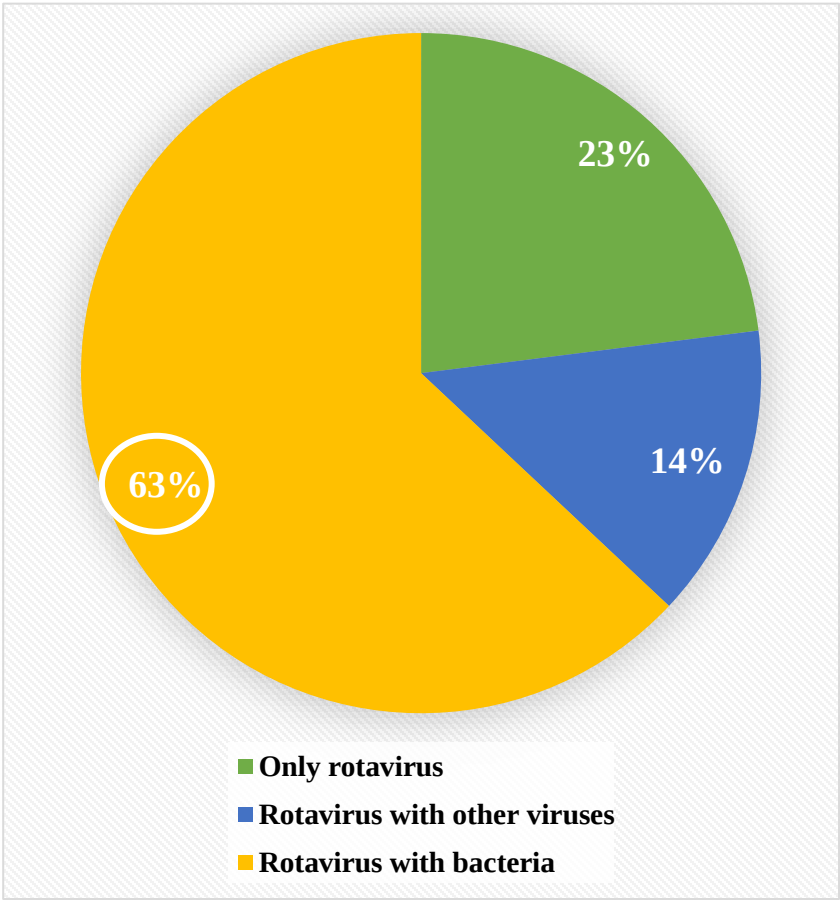
**Very high
prevalence (28%)
of rotavirus**

**Higher prevalence
(52%) of diarrheal
viruses**

Fig 4. Proportionate incidence of diarrheal viruses among children

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- Mixed infection was found in **75% (164 of 231) rotavirus** positive samples.
- Bacterial co-infection was detected among **63%** of RV-positive sample.



Pathogens	Co-infecting pathogens	Case patients no/total no. (%)
Rotavirus		231/838 (28)
	Norovirus (NoV)	19/231 (8)
	Adenovirus (AdV)	14/231 (6)
	Human bocavirus	13/231 (6)
	Both NoV and AdV	8/231 (3)
	<i>Escherichia coli</i>	112/231 (48)
	<i>Vibrio cholerae</i>	93/231 (40)
	<i>Shigella</i> spp.	43/231 (19)
	<i>Salmonella</i> spp.	17/231 (7)

Fig 5. Mixed infection of rotavirus positive samples

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- Rotavirus infection was most prevalent in children **aged 1-11 months (55%)**.
- The ratio of male to female cases was **2:1**.

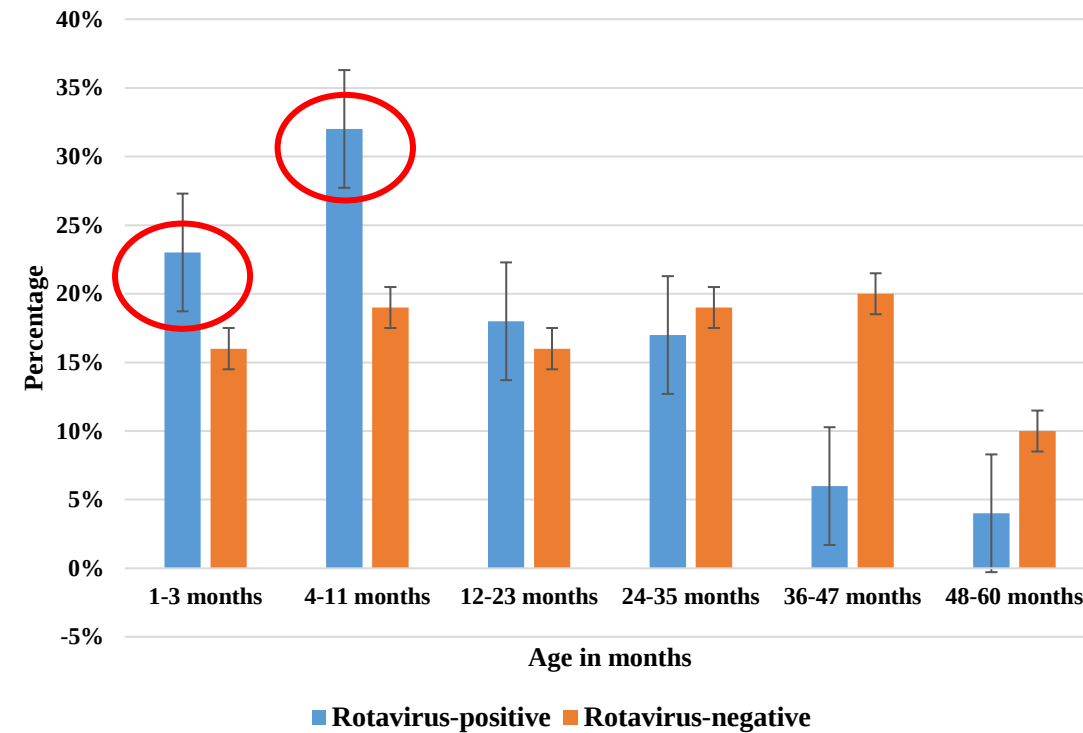


Fig 6. Age distribution of rotavirus-positive cases

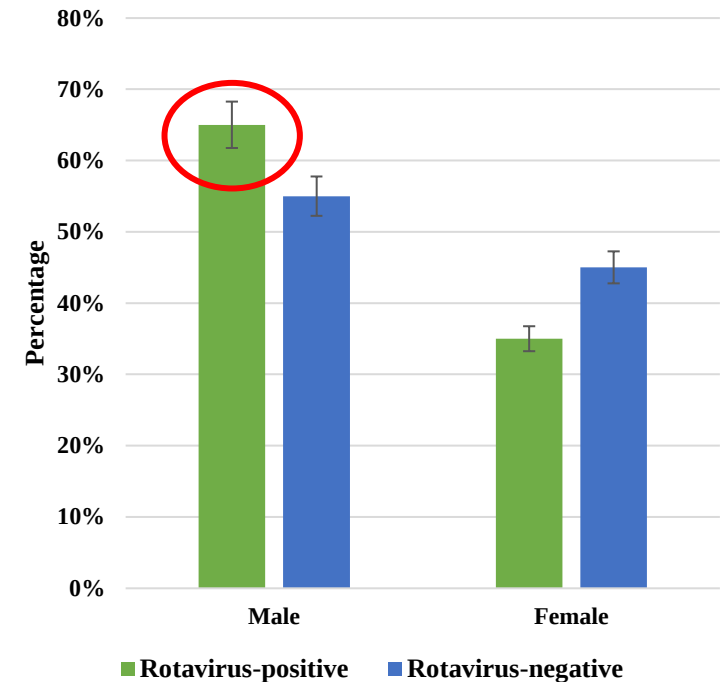
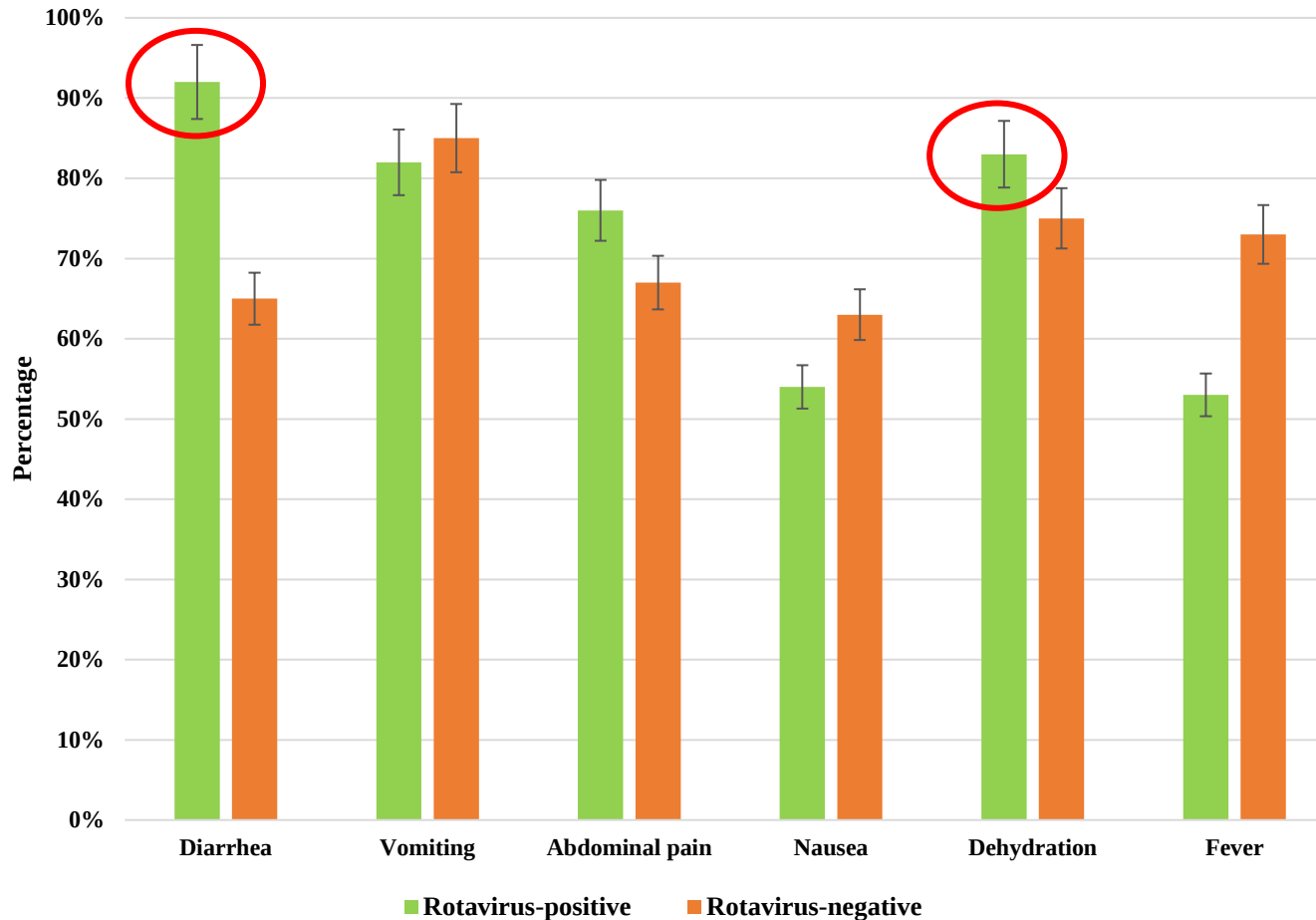


Fig 7. Gender distribution of rotavirus-positive cases

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- **Diarrhea (92%)** was the most prevalent symptom followed by **dehydration (83%)** and **vomiting (82%)**.



Similar with
previous findings

Diarrhea 92%

Vomiting 82%

Dehydration 83%

Fig 8. Distribution of clinical symptoms among children with gastroenteritis

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Table. Year-wise prevalence of rotavirus infection in Bangladesh

Year	Total Samples	No. of rotavirus positive samples	% Rotavirus positive
2014	102	17	17
2015	102	19	19
2016	105	21	20
2017	101	30	30
2018	100	27	27
2019	100	26	26
2020	101	28	28
2021	127	63	50
Total	838	231	28

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- The incidence of rotavirus was highest in 2021 (63 of 231).

A peak with 51% cases
Winter (December-January)

Rotavirus infection was
found all around the
year

Another peak in the
rainy season (14%)

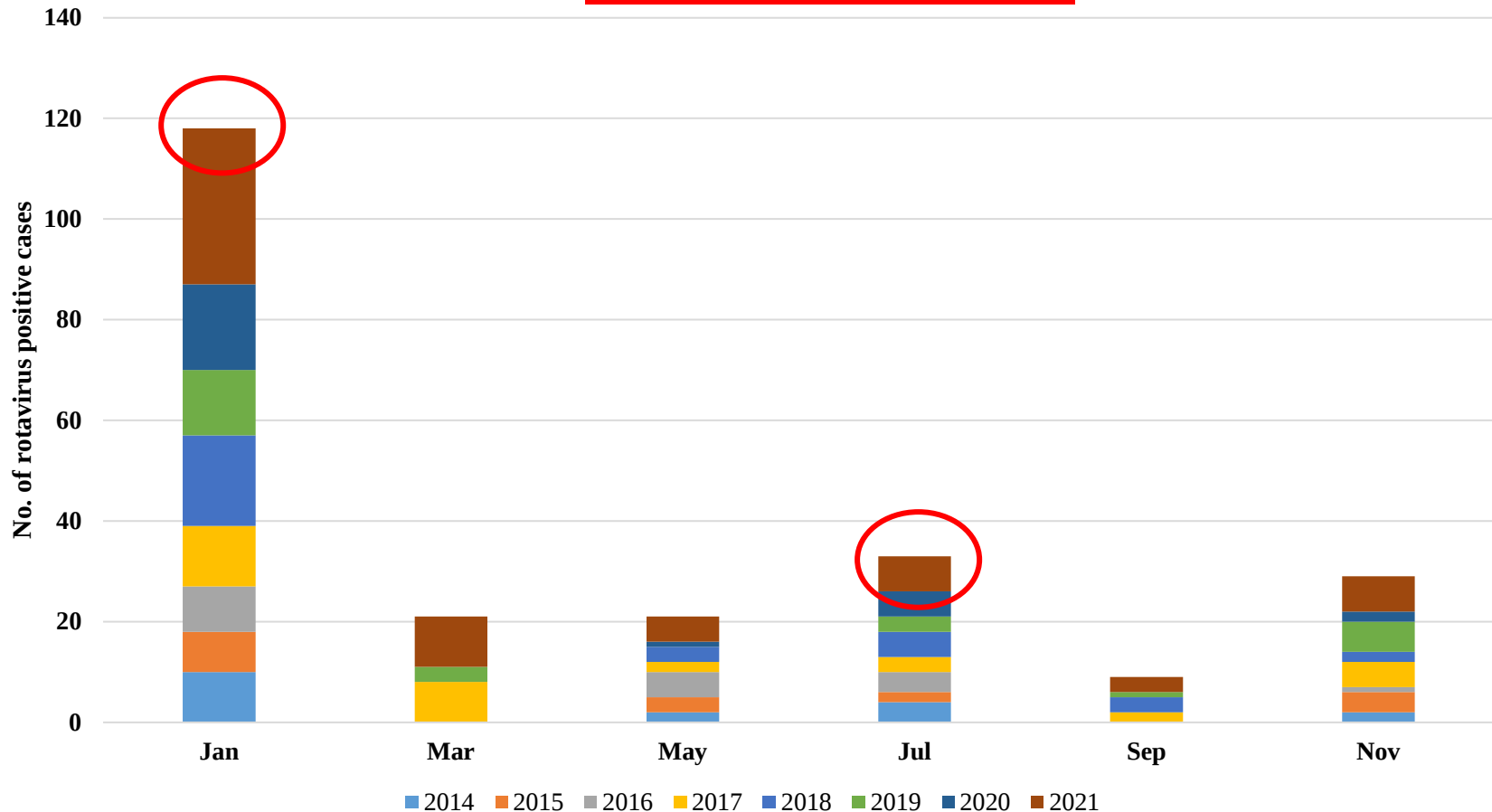


Fig 9. Seasonality of rotavirus infection among children in Bangladesh

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Great genotypic diversity
of rotavirus in
Bangladesh

G1P[8] (49%) was
consistent during 2014-21

**Prevalence of G2P[4]
(32%)** decreased from
2015 to 2021

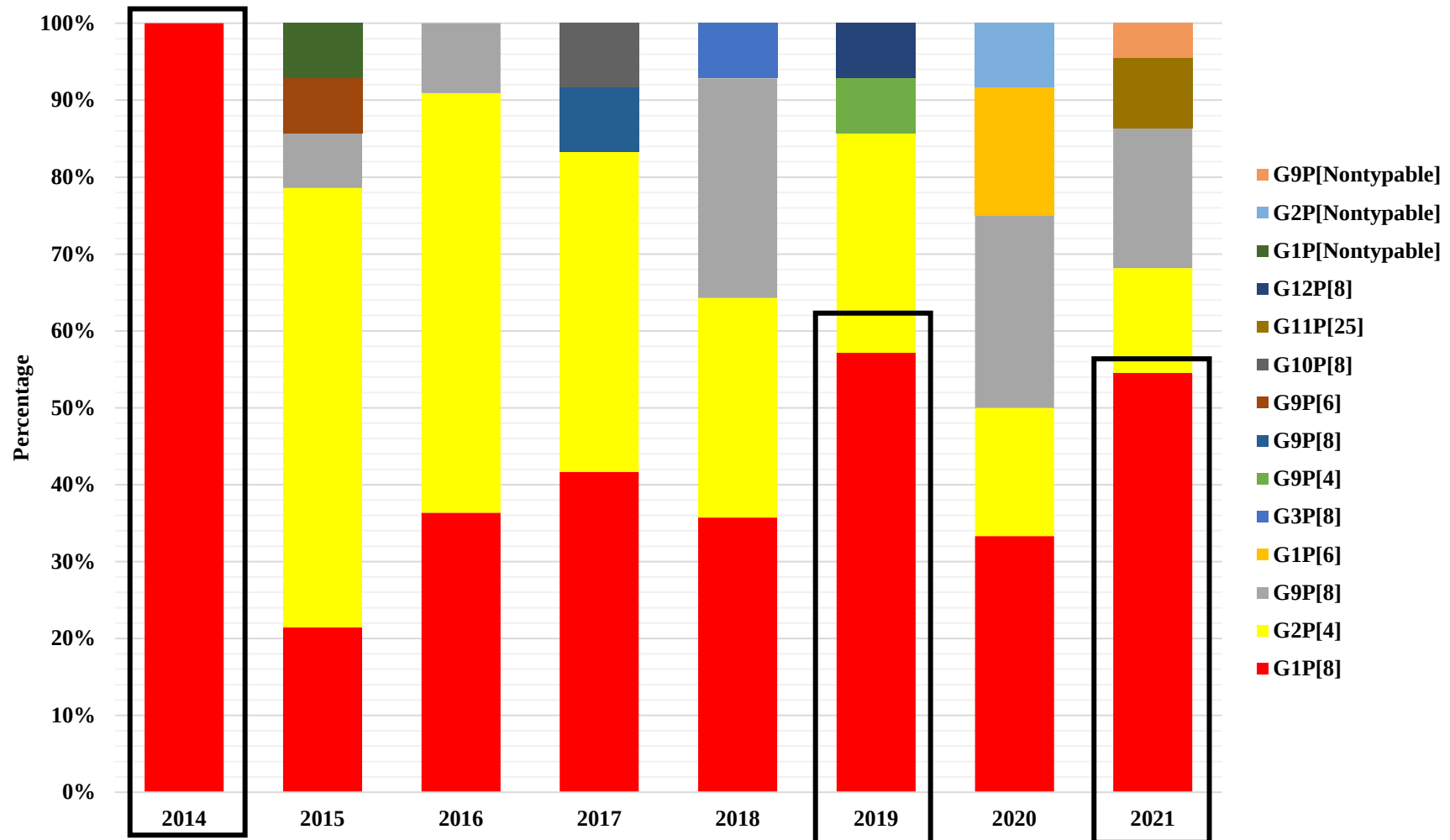


Fig 10. Genotypic distribution of rotavirus in Bangladesh during 2014-2021

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All of the rotavirus was
from Rotavirus A
(100%) genogroup

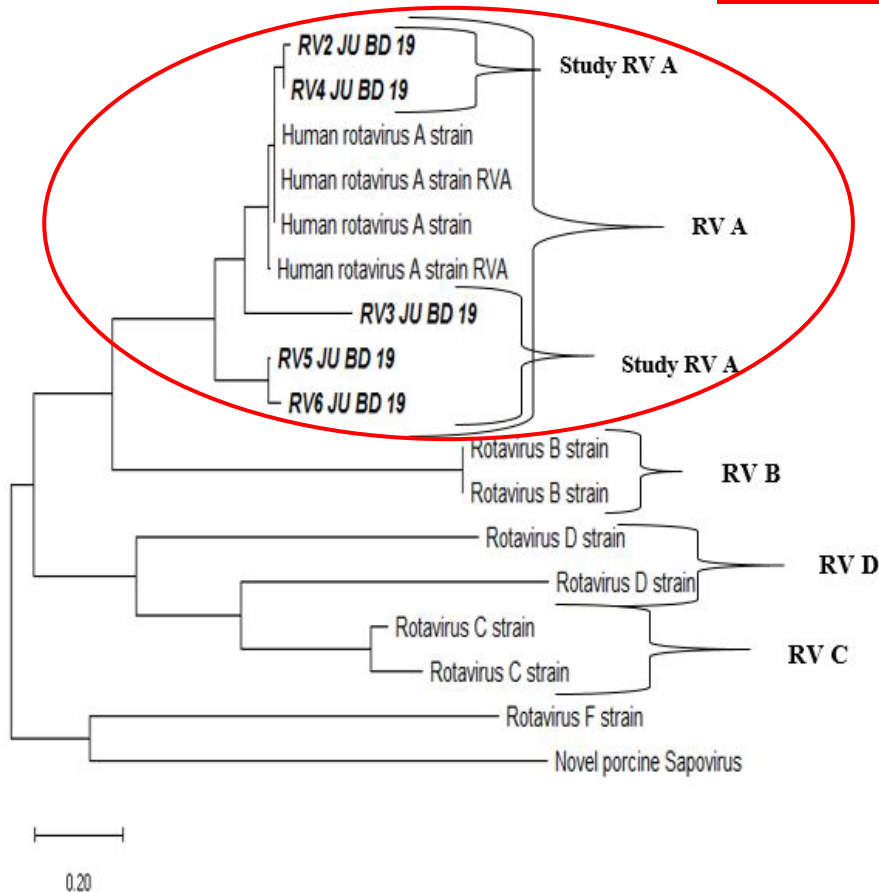


Fig 11. Phylogenetic tree of VP7 nucleotide sequence of Bangladeshi rotavirus A isolates. The scale indicated nucleotide substitutions per position. Study rotavirus A strains were indicated in italic bold in the tree.

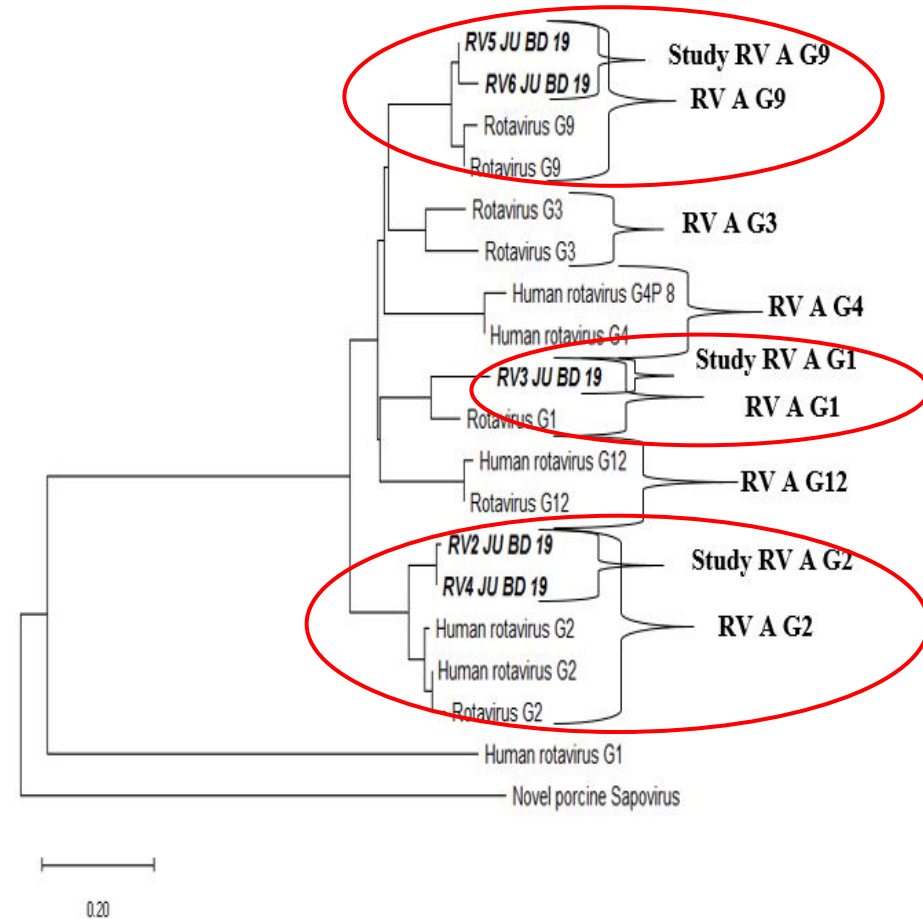


Fig 12. Phylogenetic tree of VP7 nucleotide sequence of Bangladeshi rotavirus G genotypes. The scale indicated nucleotide substitutions per position. Study rotavirus A strains were indicated in italic bold in the tree.

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- Study rotavirus were closely related with previously reported strains from **Bangladesh** and **India, USA and Korea**.

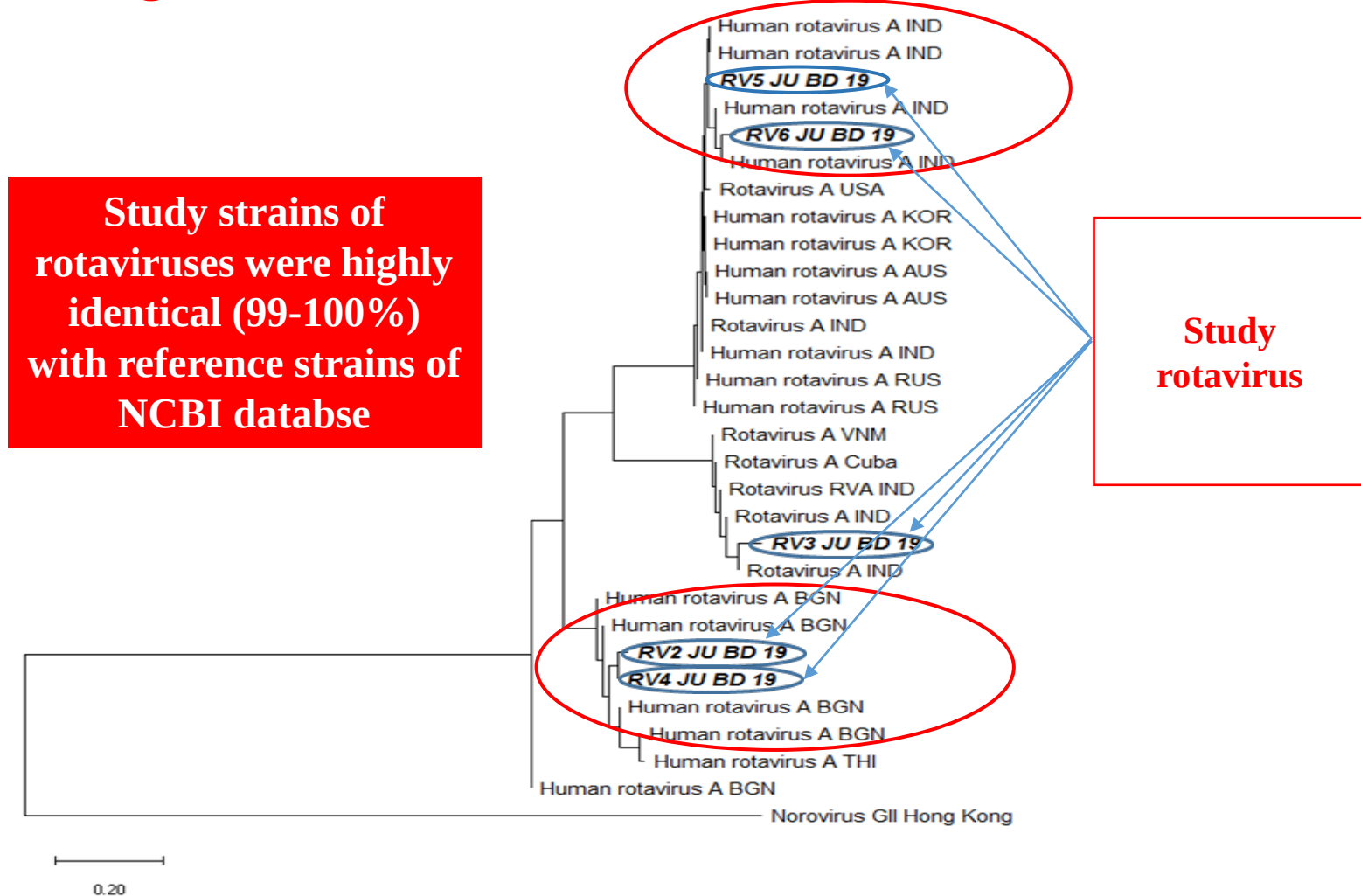
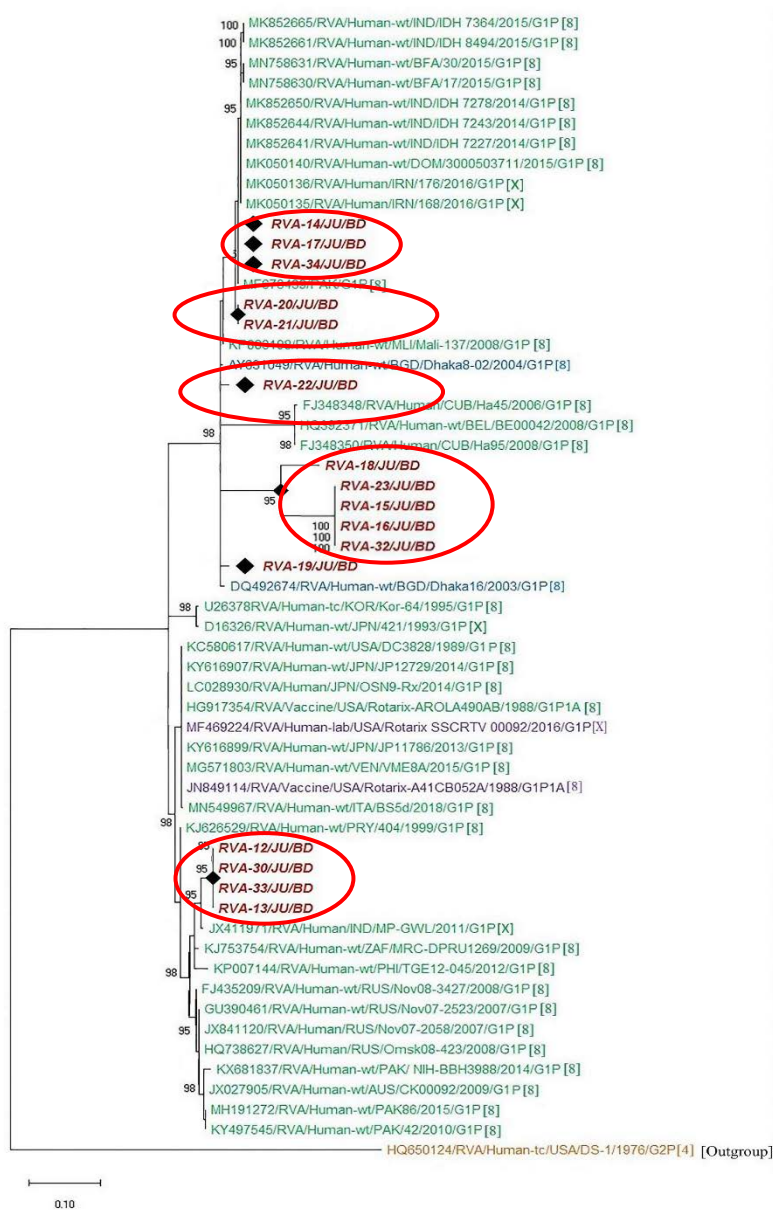


Fig 13. Phylogenetic tree of VP7 nucleotide sequence of Bangladeshi rotavirus isolates. The scale indicated nucleotide substitutions per position. Study rotavirus A strains were indicated in italic bold in the tree.



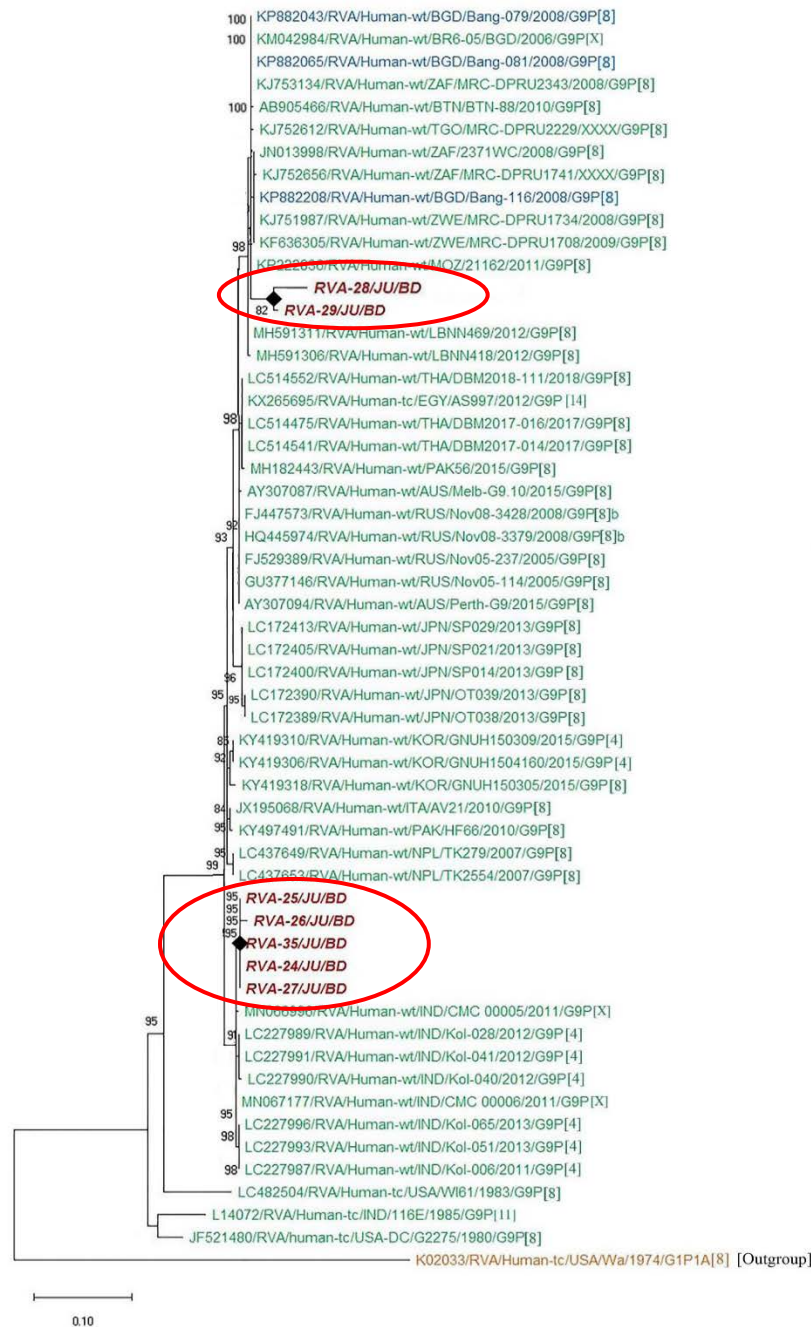
G1P[8] (49%) was the most prevalent followed by G2P[4] (32%)

100% similarity with G1P[8] of Mali, Cuba, India, Italy and Iran

99-100% similarity G2P[4] of Bangladesh, Australia and India



Fig 14 & 15. Maximum likelihood phylogenetic tree constructed from the nucleotide sequences of G1-VP7 and G2-VP7 strains and representative RVA strains with kimura-2-parameter model in **MEGA X**. Bootstrap values <60 are not shown. RVA strains sequenced in this study are represented by the red color in italic bold letter. Bangladeshi strains reported in the previous studies are shown in blue. The vaccine strains are represented by purple color, while green shading represent strains isolated all over the world.



99-100% similarity with G9P[8] of Mozambique, Lebanon, India and Nepal

G1P[8] into lineage I (73%) and IV (27)

Distributed in four sublineages in lineage I

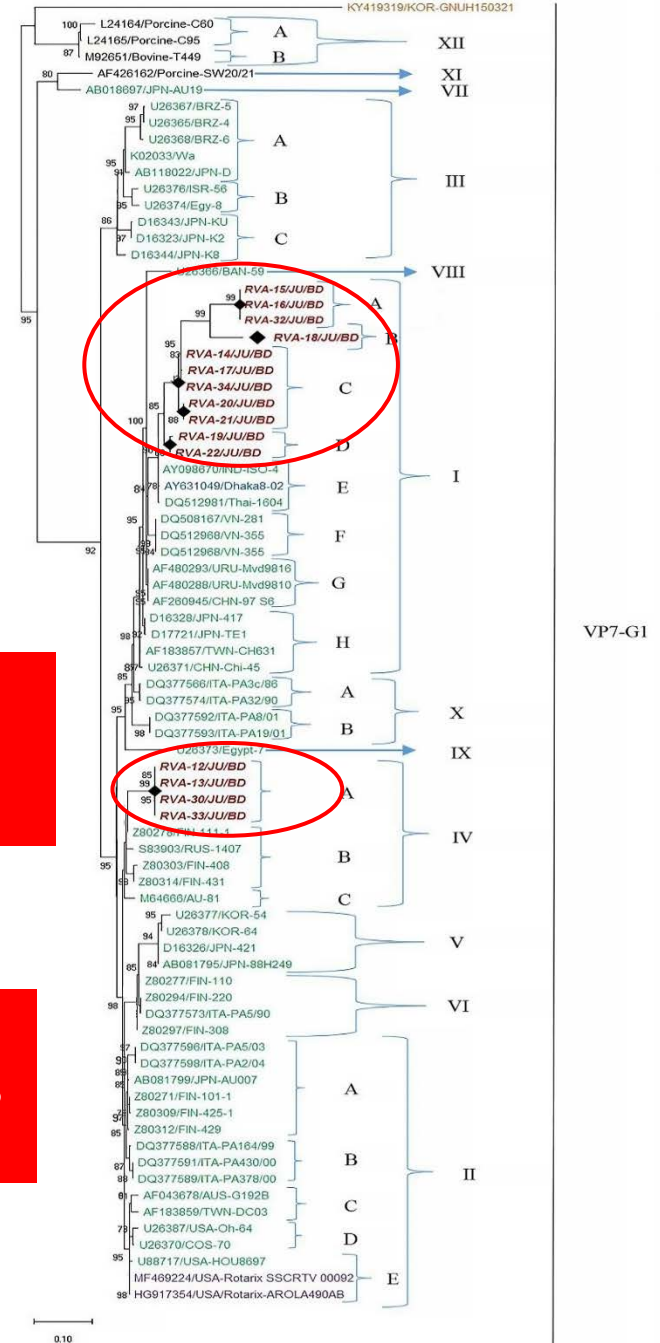


Fig 16 & 17. Maximum likelihood phylogenetic tree constructed from the nucleotide sequences of G1-VP7

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- Rapid detection of rotavirus by immunochromatography (IC) kit (IP-Noro/Rota, ImmunoProbe Co., Ltd., Saitama, Japan).
- IC kit detected **8%** (10 of 125) samples as **rotavirus positive**.



Fig 18. Fresh IC kit before the test



Fig 19. Negative test for both RV and NoV



Fig 20. RV positive test on the IC kit



Fig 21. NoV positive test on IC kit

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- For rotavirus detection, sensitivity of the IC kit was **100%** and specificity was **99.13%** against RT-PCR.

Table. Sensitivity and specificity of IC kit for rotavirus detection in stool samples

Test Result	RT- PCR		Total	Sensitivity	Specificity
IP-Noro/Rota kit	+	-		100%	99.13%
+	9	1	10		
-	0	115	115		
Total	9	116	125		

Discussion

- Very high incidence of rotavirus (**28%, 231 of 838**) was found in in this study, which is greater than previous studies in Bangladesh (Dey et al., 2009 & 2020).
- A significant level of genetic diversity of rotavirus was detected including **G1P[8], G2P[4], G1P[6], G3P[8], G9P[4], G9P[8], G9P[6], G10P[8], G11P[25], and G12P[8]**.
- **G1P[8] (49%)** was the most frequent followed by **G2P[4] (32%)** and **G9P[8] (13%)**. Notably, we reported higher diversity of rotavirus and these findings are similar with the previous studies (Dey et al., 2009 & 2020; Rahman et al., 2009).
- The predominance of genotype **G2P[4]** decreased from **8% to 3%** and **G1P[8]** increased from **8% to 12%** during 2014 to 2021.
- We detected higher incidence (**27%, 63 of 231**) of rotavirus in 2021 than any of the previous years.

Discussion

- Rotavirus infection gave **two peaks**, one in winter (**51%**) and another in the rainy season (**14%**).
- Bacterial **co-infection** was detected among **63%** of RV-positive sample which is in good agreement with previous study (Sharif *et al.*, 2021).
- Among rotavirus infected children, **Diarrhea (92%)** was the most prevalent symptom followed by **dehydration (83%)**, which is similar with previous findings from **other localities** in Bangladesh (Dey *et al.*, 2020; Rahman *et al.*, 2009).
- **Performance** of IC kit for rapid identification of rotavirus was in good agreement with previous findings (Shaha *et al.*, 2021).

Conclusion

- Still the burden of rotavirus associated children gastroenteritis is significantly high in Bangladesh.
- The genetic diversity of circulating rotavirus is also greater in the study regions.
- Inclusion of rotavirus vaccine in the national immunization program (EPI) is essential in the present situations of Bangladesh.
- **World Health Organization** and **Gavi** should take immediate and necessary steps and support the Government of Bangladesh to include rotavirus vaccine in the **immunization program**.



Acknowledgement

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- **Ming Zhang**, PhD, Associate Professor, Department of Epidemiology, University of Georgia, USA.
- **Khalid J Alzahrani**, PhD, Associate Professor, College of Applied Medical Sciences, Taif Univeristy, Saudi Arabia.

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Thank You