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**Title: Molecular Characterization of Group A Rotavirus from Rhesus Macaques (*Macaca mulatta*) at Human-Wildlife Interfaces in Bangladesh**

**Running title: Rotavirus A in Rhesus macaques**

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## Summary

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Group A rotavirus (RVA) is an important cause of diarrhea in people, especially children, and animals globally. Due to the segmented nature of the RVA genome, animal RVA strains have the potential to adapt to the human host through re-assortment with other co-infecting human viruses. Macaques share food and habitat with people, resulting in close interaction between these two species. This study aimed to detect and characterize RVA in rhesus macaques (*Macaca mulatta*) in Bangladesh. Fecal samples (N=454) were collected from apparently healthy rhesus macaques from nine different sites in Bangladesh between February and March 2013. The samples were tested by one-step, real-time, reverse transcriptase polymerase chain reaction (PCR). Four percent of samples (N=20; 95% CI 2.7-6.7%) were positive for RVA. RVA positive samples were further characterized by nucleotide sequence analysis of two structural protein gene fragments, VP4 (P genotype), and VP7 (G genotype). G3, G10, P[3] and P[15] genotypes were identified and were associated as G3P[3], G3P[15] and G10P[15]. The phylogenetic relationship between macaque RVA strains from this study and previously reported human strains indicates possible transmission between humans and macaques in Bangladesh. To our knowledge this is the first report of detection and characterization of rotaviruses in rhesus macaques in Bangladesh. These data will not only aid in identifying viral sharing between macaques, human and other animals, but will also improve the development of mitigation measures for the prevention of future rotavirus outbreaks.

**Keywords:** Genotyping, Group A Rotavirus, *Macaca mulatta*, Phylogenetic analysis, Rhesus macaque.

## Introduction

Group A rotavirus (RVA) is the leading etiological agent responsible for severe acute diarrhea in children aged less than 5 years worldwide. Rotavirus infection results in an estimated 611,000 deaths annually, mostly in low income countries (Tate et al., 2012). RVA, a member of the family *Reoviridae*, is a triple layered, non-enveloped virus particle, with 11 segments of double-stranded RNA genome encoding six structural viral proteins (VP1-VP4, VP6, and VP7) and six nonstructural proteins (NSP1–NSP6) (Hu et al., 2012). Structurally, the viral capsid has three layers- the VP2 protein composes the innermost layer, VP6 the middle layer, VP4 and VP7 compose the outermost layer. The VP4 (protease sensitive) and VP7 (glycoprotein) trigger the production of antibodies in

host. These two proteins VP4 and VP7 also determine the P genotype and G genotype of the virus, respectively (Matthijnsens et al., 2010; Sai et al., 2013). At present, RVA genotypes are studying as the genotype constellation based on the 11 genomes. Thirty-six distinct G genotypes and 51 P genotypes have been identified (RCWG).

The segmented nature of the virus genome provides the opportunity for genetic reassortment that may lead to a new combination of genes with potentially altered viral infectivity and pathogenesis (Maunula and von Bonsdorff, 2002). The majority of ongoing research on rotaviruses (RVs) is focused on the reassortment of VP7 and VP4. Different combinations of G and P genotypes have been detected in humans (Saudy et al., 2016; Aydin and Aktaş, 2017). The most common G types, which are found globally in humans are G1, G2, G3 and G4 in conjunction with P[8] or P[4], followed by G9 associated with P[8] or P[6]. P[8]G1 has been found mainly in Australia, North America and Europe whereas G8 has been found primarily in Africa. P[6] is the most frequently detected P type and unusual combinations such as G8P[6] or G8P[4] have also been identified in people in Africa (Santos and Hoshino, 2005). G5, G8 and G10 have been identified recently in different parts of the world, including in India, Ghana, Australia, Argentina, Paraguay, and Malawi (Hoshino et al., 2003). Recently, the G12 genotypes has been re-emerged in humans in the United States (Wylie et al., 2017), Spain (Cilla et al., 2014) and Tunisia (Moussa et al., 2017). The remarkable diversity of human RVA strains is associated with three major evolutionary mechanisms: the accumulation of point mutations leading to antigenic drift; reassortment of cognate genome segments promoting antigenic shift; and the reassortment of genes in strains found in animals that could introduce new antigen types into humans (Martella et al., 2010; Matthijnsens et al., 2010).

Experimental models have identified RVA in numerous animal species, which are host-specific (Brugere-Picoux and Tessier, 2010). Despite host-virus specificity, zoonotic transmission has been identified by cross-infection where there is a close genetic relationship between certain human and animal rotaviruses (Brugere-Picoux and Tessier, 2010). The potential for cross-species transmission of viral pathogens, in general, exists where humans and non-human primates (NHP) come into contact and has been documented between humans and several species of NHP in a variety of contexts and in diverse geographic areas (Schillaci et al., 2005; Jones-Engel et al., 2008; Epstein and Price, 2009).

Human-NHP interaction is common in Asia, particularly in Bangladesh, where humans and NHP have lived sympatrically for centuries (Sha et al., 2009; Engel et al., 2010). The contexts of contact between humans and NHPs in Bangladesh are a microcosm of what is seen throughout much of South and Southeast Asia. Their population densities vary from a single troop of less than 20 animals in urban areas like Dhamrai and Bormi, Bangladesh to multiple troops (up to 200 individuals) roaming through the villages (Feeroz et al., 2013). In Bangladesh, free-ranging rhesus macaques are found in a broad range of habitats, from protected forests and nature preserves, where there is little overlap with humans, to rural villages, gardens, and religious sites and to densely populated cities. This synanthropic monkey particularly thrives in human-altered habitats (Oberste et al., 2013).

This study was of interest because rhesus macaques in Bangladesh live in close proximity to human populations and thus there is the possibility of interspecies transmission of rotaviruses. The present study reinforces the hypothesis that human rotaviruses might be able to cross the species barriers, and the lack of systematic surveillance of rotavirus infection in animals hinders the ability to establish firm epidemiologic connection.

## **Materials and Methods**

### ***Ethical statement***

The study was conducted under ethical approval from the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b; protocol 2008-074) and UC Davis (protocol 16048)

### ***Specimen collection and preparation***

In Bangladesh, rhesus macaques are found in both forest habitats and urban areas. This study focused on macaques from nine sites in Bangladesh (Figure 1). The macaques were baited with food (e.g. bananas, breads, puffed rice etc.) and monitored at the feeding site for defecation. Approximately 500µl of feces per animal was collected from macaques from February to March 2013. The health status, sex and age of each macaque was visually assessed. A convenience sampling method was used for sample collection. The fecal samples were collected in a tube containing lysis buffer immediately following defecation. The samples were preserved at -80°C until laboratory testing. All precautions were taken to minimize the risk of exposure of our staff to different zoonotic pathogens and to

minimize the risk of anthropogenic transmission back to the macaques. Appropriate PPE was used and biosafety measures were taken during sampling and sample processing/testing.

### ***RNA extraction and qRT-PCR***

The samples were transported to the Virology Laboratory at icddr,b. Total viral nucleic acid was extracted from the fecal samples using magnetic particle based InviMag® Virus DNA/RNA Mini Kit (STRATEC Molecular GmbH, Germany) according to the manufacturer's instructions. The detection of RVA was carried out by real time, one-step, reverse transcriptase polymerase chain reaction (qRT-PCR) according to the procedure described by Jothikumar et al. (2009). qRT-PCR was carried out using the QIAGEN® One-step RT-PCR Kit (QIAGEN, Germany) according to the manufacturer's instructions. PCR positive samples were then assayed with a conventional RT-PCR for the amplification of VP7 and VP4 genes fragments using the consensus primer pairs Beg9/End9 (5'-ATG TAT GGT ATT GAA TAT ACC AC-3'/ 5'-AAC TTG CCA CCA TTT TTT CC-3') and Con2/Con3 (5'-ATT TCG GAC CAT TTA TAA CC-3'/ 5'-TGG CTT CGC TCA TTT ATA GAC A-3'), respectively (Gentsch et al., 1992; Rahman et al., 2007).

Denaturation of viral dsRNA was carried out by heating at 95°C for 5 minutes and subsequent rapid cooling on ice prior to actual reverse transcription reaction. After that the RT-PCR was carried out with an initial reverse transcription step at 50°C for 30 min, initial PCR activation step at 95°C for 15 min, followed by 40 cycles of amplification (30s at 94°C, 30s at 50°C, 1 min at 72°C) and final extension at 72°C for 7 min in a thermal cycler (T<sub>3000</sub> Thermocycler, Biometra, USA). PCR products were run on a 1.5% agarose gel, stained with ethidium bromide and visualized under a UV transilluminator (Bio-Rad Laboratories, CA, USA).

### ***Nucleotide sequencing***

Nucleotide sequencing of amplified PCR products was carried out in an automated ABI 3500 xL Genetic Analyzer (Applied Biosystem, Foster City, CA, USA) and Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, Foster City, CA, USA) as per kit protocol. The chromatogram sequencing files were inspected using Chromas 2.3 (Technelysium, Helensvale, Australia). Sequences were compared with existing GenBank database using the BLASTN program at the National Center

for Biotechnology Information (BLAST). The sequences obtained from the study were submitted to GenBank under the accession numbers MK610258 through MK610264 and MK628594 through MK628596.

### ***Analysis***

The samples were presented as frequency and percentage along with 95% confidence interval (CI) according to different variables. Study sites were categorized as peri-urban, urban and rural areas based on geography and macaques were classified as adult, sub-adult and juvenile based on the PREDICT Operating procedures Non-Human primate sampling.

To perform phylogenetic analysis, sequences were edited and analyzed using BioEdit (Hall TA, 1999) and MEGA version 6.0 (Tamura et al., 2013). The evolutionary relatedness was inferred by using the maximum likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). Bootstrap values (n = 1000 replicates) are shown at branch nodes and the scale bar represents the number of changes per nucleotide position.

### **Results**

#### ***Detection of Rotavirus***

A total of 20 (4%; 95% Confidence Interval/CI: 2.7-6.7) of 454 samples collected from apparently healthy individual rhesus macaques were positive for RVA by one-step qRT-PCR assay. The prevalence of RVA was highest in adults (4.9%; 95% CI: 2.6-8.2), male (5.0%; 95% CI: 2.5-8.8) and peri-urban areas (5.3%; 95% CI: 2.2-10.7) (Table 1).

#### **Distribution of G and P genotypes**

Seven (35%) of the positive samples were able to be genotyped. Five strains were detected at the same place, Bandar, Narayanganj. G3 and G10 genotypes were identified within the seven samples among which G3 was most prevalent (6/7; 85.7%; 95% CI:4.1-99.6), while only one G10 was detected (1/7; 14.3%; 95% CI:0.3-57.8). Among the seven samples genotyped as G3/G10, only three

samples were also successfully genotyped for the VP4 protein (P genotype), with the final resultant combinations of G3P[3], G3P[15] and G10P[15] (Table 2).

### ***Phylogenetic Analysis***

Nucleotide sequence similarity of the VP7 gene fragments of the strains detected in Bangladesh revealed that 5 of the 6 G3 genotype RVA strains from macaques in Bangladesh (PGB-690, PGB-691, PGB-692, PGB-700, and PGB-701) were identical to each other and belonged to RVA genotype G3 lineage 1. The strains were close relatives of the simian-like bat, human, simian-like human and alpaca RVA strains with 93% sequence similarity at the nucleotide level and clustered tightly in the phylogenetic tree (Figure 2). However, the nucleotide sequence similarity of these strains with other relatives of RVA genotype G3 lineage 1 ranged from 85-86%. In contrast, the strain PGB-980 isolated from Madaripur clusters with feline, canine and feline/canine-like G3 RVA. The strains had 97-99% nucleotide sequence similarity of its VP7 gene fragment with that of the feline/canine strains within G3 lineage 3 (Figure 2). PGB-876 was the only strain which belonged to genotype G10 and clustered closely with human and bovine RVA strains with 95.5-98.5% nucleotide similarity in the VP7 gene fragment. This strain also had 91-95.5% nucleotide sequence similarity with Bangladeshi strains isolated in 2009-2010 from goat and cattle and within the same cluster as lineage 5 (Figure 3).

Two P[15] rotavirus genotypes, G10 and G3, PGB-876 and PGB-980, respectively, clustered closely with bovine RVA strains isolated in India and Bangladesh and were distantly related to the human and caprine P[15] RVA strains (Figure 4). The highest similarity was found with a Bangladeshi strain RVA/Cattle/BGD/GBNL0014/2009/G10P15, which had a 97-100% nucleotide similarity. The P[15] strains also had similarities (96%) with the strains isolated from a cow (GenBank: FJ798615) in India (Figure 4). The VP4 gene fragment of PGB-692 clustered with the simian and simian-like RVA group with a nucleotide similarity of 85% (84.8-86.5%) as compared to the VP4 gene fragments (PGB-876 and PGB-980) that clustered with bovine RVA strains from India (Figure 5).

### **Discussion**

Rhesus macaques are native to Bangladesh and widely distributed throughout the country (Hasan et al., 2013). This study identifies the presence of RVA in rhesus macaques of Bangladesh and investigates the origin and ancestry of the virus. Macaques have a tendency to acquire enteric viruses

from humans (Oberste et al., 2013) and rotaviruses have been identified in samples from them previously (Jiang et al., 2004). Macaques thrive in the human-altered habitats within villages, urban areas, and religious sites found throughout Bangladesh. The close association of macaque and human populations in Bangladesh provides conditions favorable for the interspecies transmission of infectious agents (Oberste et al., 2012). Habitat destruction and anthropogenic changes to the environment have dramatically reduced the ability of macaques to naturally disperse and have likely increased human-macaque interactions (Altizer et al., 2018). Therefore, it is not surprising that we have detected rotaviruses from the rhesus macaque population of Bangladesh.

### **RVA prevalence**

The prevalence of RVA in macaques in the present study is lower than a previous study in a research center of United States (Jiang et al., 2004). Macaques can become infected with rotavirus from various sources, such as contaminated food and water (Kaur et al., 2008). A common behavior of rhesus macaques is swimming (Dunbar, 1989), that habit predisposes them to rotavirus infection if the water is contaminated by the virus. Most of the food that macaques consume are from human sources, either as direct handouts or from agricultural sources, which can lead to disease transmission (Altizer et al., 2018). Additionally, macaques are known to consume human excreta, which is primarily the source of rotavirus in the environment (Richard et al., 1989; Kaur et al., 2008). In our study, all of the rotaviruses detected were in rhesus macaques whose home ranges overlapped with humans. Previous studies have demonstrated that non-human primates living in close association with humans are at risk of infection with human pathogens (Epstein and Price, 2009). Macaque in Bangladesh, especially in urban settings with high human density and poor waste management, would likely be at high risk for acquiring human enteric pathogens. Our findings support the zoonothroponotic transmission of RVA to macaques in Bangladesh.

### **G and P genotypes**

By applying PCR-based direct sequence techniques in this study, we have successfully detected and characterized rotaviruses using the VP7 and VP4 genes. Among the positive samples, only seven were able to be genotyped. However, the detection of six G3 strains of rotavirus out of seven RVA positive samples of macaques in Bangladesh is very interesting, because the G3 strain was last reported in the human population in Bangladesh in 2001 (Rahman et al., 2007). Until early 1990s, G3 genotype was



one of the most important RVA genotypes in humans. The live attenuated human rotavirus vaccine, RotaTeq® (Merck & Co., Whitehouse Station, NJ, USA) even includes the G3 strain, and is currently used in more than 90 countries in the world including Bangladesh (Afrad et al., 2013). The vaccine strain can be shed in human stool between days 4-7 following the initial dose (Hsieh et al., 2014) and horizontal transmission of vaccine strain occurs very frequently (Payne et al., 2010). The vaccine strain may be transmitted to macaques as well, due to their frequent interactions with humans or through the contamination of food sources or water reserves by human waste and subsequent entry to macaque population. Thus, any emergence of a G3 strain in future may lead to a possible correlation with the rotavirus strains reported here.

Although G3P[3] has been reported in animals from India (GenBank KC416959.1), China (GenBank: KX814954), Japan (GenBank: AB055967) and Brazil (GenBank: KR106161), it has only been reported as a human infection in Japan (Matthijnsens et al., 2008) and China (GenBank: KU597745). This is the first report of G3P[3] in Bangladesh. It is possible that the reported illegal trade of cattle that occurs between India and Bangladesh (Banerjee et al., 1999), may facilitate the movement of rotaviruses between the countries. These bovid hosts may come into contact with macaques during transport or if the cow is shedding the virus, feces can contaminate food or water that the macaques consume, permitting the transmission of RVA.

The G3P[15] strain was found in one rhesus macaque sample in the study. This is a rare combination which has not been reported in any species previously. G10P[15] serotype is also an unusual combination though it was previously detected in cattle from India in 2007 (Rajendran and Kang, 2014). With the potential to spill over into humans, this presents a possible public health threat (Komoto et al., 2014). G10, P3 and P15 have not previously been detected in Bangladesh.

### **Phylogenetic analysis**

The phylogenetic analysis revealed the ancestral relatedness of different genotypes of RVA sequenced from macaques in Bangladesh. Five of the six G3 genotype RVA strains were identical in this study. The G3 strains of this study have similarity with feline/canine-like, human, simian and simian-like RVAs previously detected in humans and other animals. Since the samples were collected from free-living, apparently healthy macaques, the finding of this study suggests that possible asymptomatic infection of RVA G3 strains in the rhesus macaques in Bangladesh may have a long duration. The

wide host breadth of RVA strains indicated the potential of RVAs for interspecies transmission. Only one G10 strain was identified in the study, this strain is uncommon in human (Luchs and Timenetsky Mdo, 2016; Do et al., 2017) but are frequently found in animals, particularly in cattle, pigs, and lambs (Komoto et al., 2014; Rajendran and Kang, 2014; Madadgar et al., 2015; Ennima et al., 2016; Mohamed et al., 2017). G10P[15] is an unusual combination but has previously been reported in a calf in India (Rajendran and Kang, 2014). This indicates possible interspecies transmission among animals as macaques also share food and water sources with livestock populations. This is the first report on the characterization of rotaviruses from rhesus macaques in Bangladesh.

### **Conclusions**

We identified rotavirus genotype strains of G3, G10, P[3] and P[15] from rhesus macaques in three districts of Bangladesh. The study results indicate possible interspecies transmission and/or reassortment of RVA between humans and macaques. The close proximity and interactions between humans and macaques may facilitate anthroozoonotic or zoonotic transmission. Moreover, this strengthens the evidence for reassortment events between animal and human rotavirus strains, which could result novel infectious strains of rotavirus in humans or macaques.

As the generation of atypical or novel strains is likely more common in developing countries (Luchs and Timenetsky Mdo, 2016), surveillance for human rotavirus strains can play a crucial role in understanding rotavirus epidemiology. The role of vaccine strains in the generation of novel genotypes should also be investigated further, as environmental contamination of human and animal waste creates opportunity for interspecies transmission and viral evolution. Future studies should be aimed to identify methods to limit the interspecies transmission in order to reduce viral evolution and decreasing the risk to child health.

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**Conflict of interest:** None.

## References

- Afrad, M. H., Rahman, M. Z., Matthijssens, J., Das, S. K., Faruque, A., Azim, T., & Rahman, M. (2013). High incidence of reassortant G9P[4] rotavirus strain in Bangladesh: Fully heterotypic from vaccine strains. *Journal of Clinical Virology*, 58, 755-756. doi: 10.1016/j.jcv.2013.09.024
- Altizer, S., Becker, D. J., Epstein, J. H., Forbes, M. K., Gillespie, T. R., Hall, R. J., Hawley, D. M., Hernandez, S. M., Martin, L. B., Plowright, R. K., Satterfield, D. A., & Streicker, D. G. (2018). Food for contagion: synthesis and future directions for studying host-parasite responses to resource shifts in anthropogenic environments. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*, 373, 1745. <https://doi.org/10.1098/rstb.2017.0102>
- Aydin, H., & Aktaş, O. (2017). Rotavirus genotypes in children with gastroenteritis in Erzurum: first detection of G12P[6] and G12P[8] genotypes in Turkey. *Gastroenterology Review*, 12, 122-127. doi: 10.5114/pg.2016.59423
- Banerjee, P., Hazarika, S., Hussain, M., & Samaddar, R. (1999). Indo-Bangladesh cross-border migration and trade. *Economic and Political Weekly*, 34, 2549-2551.
- Basic local alignment search tool - BLAST. Retrieved from <http://www.ncbi.nlm.nih.gov/blast> (accessed on 10 September, 2019).
- Brugere-Picoux, J., & Tessier, P. (2010). Viral gastroenteritis in domestic animals and zoonoses. *Bulletin de l'Academie de Medecine*, 194, 1439-1449.
- Cilla, G., Montes, M., & Arana, A. (2014). Rotavirus G12 in Spain: 2004-2006. *Enfermedades Infecciosas y Microbiología Clínica*, 32, 405. doi: 10.1016/j.eimc.2014.01.012
- Do, L. P., Kaneko, M., Nakagomi, T., Gauchan, P., Agbemabiese, C. A., Dang, A. D., & Nakagomi, O. (2017). Molecular epidemiology of Rotavirus A, causing acute gastroenteritis hospitalizations among children in Nha Trang, Vietnam, 2007-2008: Identification of rare

G9P[19] and G10P[14] strains. *Journal of Medical Virology*, 89, 621-631.  
<https://doi.org/10.1002/jmv.24685>

Dunbar, D. C. (1989). Locomotor behavior of rhesus macaques (*Macaca mulatta*) on Cayo Santiago. *Puerto Rico Health Sciences Journal*, 8, 79-85.

Engel, G., O'Hara, T. M., Cardona-Marek, T., Heidrich, J., Chalise, M. K., Kyes, R., & Jones-Engel, L. (2010). Synanthropic primates in Asia: potential sentinels for environmental toxins. *American Journal of Physical Anthropology*, 142, 453-460. <https://doi.org/10.1002/ajpa.21247>

Ennima, I., Sebbar, G., Harif, B., Amzazi, S., Loutfi, C., & Touil, N. (2016). Isolation and identification of group A rotaviruses among neonatal diarrheic calves, Morocco. *BMC Research Notes*, 9, 261. <https://doi.org/10.1186/s13104-016-2065-8>

Epstein, J. H., & Price, J. T. (2009). The significant but understudied impact of pathogen transmission from humans to animals. *The Mount Sinai Journal of Medicine*, 76, 448-455.  
<https://doi.org/10.1002/msj.20140>

Feeroz, M. M., Soliven, K., Small, C. T., Engel, G. A., Andreina Pacheco, M., Yee, J. L., Wang, X., Kamrul Hasan, M., Oh, G., Levine, K. L., Rabiul Alam, S. M., Craig, K. L., Jackson, D. L., Lee, E. G., Barry, P. A., Lerche, N. W., Escalante, A. A., Matsen Iv, F. A., Linial, M. L., & Jones-Engel, L. (2013). Population dynamics of rhesus macaques and associated foamy virus in Bangladesh. *Emerging Microbes & Infections*, 2, e29. doi: 10.1038/emi.2013.23

Gentsch, J., Glass, R., Woods, P., Gouvea, V., Gorziglia, M., Flores, J., Das, B., & Bhan, M. (1992). Identification of group A rotavirus gene 4 types by polymerase chain reaction. *Journal of Clinical Microbiology*, 30, 1365-1373. PMID:1320625

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series*, 41(41), 95-98.

Hasan, M. K., Aziz, M. A., Alam, S. R., Kawamoto, Y., Engel, L. J., Kyes, R. C., Akhtar, S., Begum, S., & Feeroz, M. M. (2013). Distribution of Rhesus Macaques (*Macaca mulatta*) in Bangladesh: inter-population variation in group size and composition. *Primate Conservation*, 26, 125-132. <https://doi.org/10.1896/052.026.0103>

- Hoshino, Y., Jones, R. W., Ross, J., & Kapikian, A. Z. (2003). Construction and characterization of rhesus monkey rotavirus (MMU18006)-or bovine rotavirus (UK)-based serotype G5, G8, G9 or G10 single VP7 gene substitution reassortant candidate vaccines. *Vaccine*, 21, 3003-3010. [https://doi.org/10.1016/S0264-410X\(03\)00120-8](https://doi.org/10.1016/S0264-410X(03)00120-8)
- Hsieh, Y. C., Wu, F. T., Hsiung, C. A., Wu, H. S., Chang, K. Y., & Huang, Y. C. (2014). Comparison of virus shedding after lived attenuated and pentavalent reassortant rotavirus vaccine. *Vaccine*, 32, 1199-1204. <https://doi.org/10.1016/j.vaccine.2013.08.041>
- Hu, L., Crawford, S. E., Hyser, J. M., Estes, M. K., & Prasad, B. V. (2012). Rotavirus non-structural proteins: structure and function. *Current Opinion in Virology*, 2, 380-388. <https://doi.org/10.1016/j.coviro.2012.06.003>
- Jiang, B., McClure, H. M., Fankhauser, R. L., Monroe, S. S., & Glass, R. I. (2004). Prevalence of rotavirus and norovirus antibodies in non-human primates. *Journal of Medical Primatology*, 33, 30-33. <https://doi.org/10.1111/j.1600-0684.2003.00051.x>
- Jones-Engel, L., May, C. C., Engel, G. A., Steinkraus, K. A., Schillaci, M. A., Fuentes, A., Rompis, A., Chalise, M. K., Aggimarangsee, N., & Feeroz, M. M. (2008). Diverse contexts of zoonotic transmission of simian foamy viruses in Asia. *Emerging Infectious Diseases*, 14, 1200. <https://dx.doi.org/10.3201%2F1408.071430>
- Jones-Engel, L., Engel, G. A., Schillaci, M. A., Lee, B., Heidrich, J., Chalise, M., & Kyes, R. C. (2006). Considering human-primate transmission of measles virus through the prism of risk analysis. *American Journal of Primatology*, 68, 868-879. <https://doi.org/10.1002/ajp.20294>
- Jothikumar, N., Kang, G., & Hill, V. R. (2009). Broadly reactive TaqMan assay for real-time RT-PCR detection of rotavirus in clinical and environmental samples. *Journal of Virological Methods*, 155, 126-131. <https://doi.org/10.1016/j.jviromet.2008.09.025>
- Kaur, T., Singh, J., Tong, S., Humphrey, C., Clevenger, D., Tan, W., Szekely, B., Wang, Y., Li, Y., & Alex Muse, E. (2008). Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (*Pan troglodytes*) at Mahale Mountains National Park, Western Tanzania. *American Journal of Primatology*, 70, 755-765. <https://doi.org/10.1002/ajp.20565>

- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-120. <https://doi.org/10.1007/BF01731581>
- Komoto, S., Pongsuwanna, Y., Ide, T., Wakuda, M., Guntapong, R., Dennis, F. E., Haga, K., Fujii, Y., Katayama, K., & Taniguchi, K. (2014). Whole genomic analysis of porcine G10P[5] rotavirus strain P343 provides evidence for bovine-to-porcine interspecies transmission. *Veterinary Microbiology*, 174, 577-583. <https://doi.org/10.1016/j.vetmic.2014.09.033>
- Luchs, A., & Timenetsky Mdo, C. (2016). Group A rotavirus gastroenteritis: post-vaccine era, genotypes and zoonotic transmission. *Einstein (Sao Paulo)*, 14, 278-287. <http://dx.doi.org/10.1590/S1679-45082016RB3582>
- Madadgar, O., Nazaktabar, A., Keivanfar, H., Zahraei Salehi, T., & Lotfollah Zadeh, S.(2015). Genotyping and determining the distribution of prevalent G and P types of group A bovine rotaviruses between 2010 and 2012 in Iran. *Veterinary Microbiology*, 179, 190-196. <https://doi.org/10.1016/j.vetmic.2015.04.024>
- Martella, V., Bányai, K., Matthijnssens, J., Buonavoglia, C., & Ciarlet, M. (2010). Zoonotic aspects of rotaviruses. *Veterinary Microbiology*, 140, 246–255. <https://doi.org/10.1016/j.vetmic.2009.08.028>
- Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S. M., Palombo, E. A., Iturriza-Gómara, M., Maes, P., & Patton, J. T. (2008). Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. *Journal of Virology*, 82, 3204-3219. DOI: 10.1128/JVI.02257-07
- Matthijnssens, J., Heylen, E., Zeller, M., Rahman, M., Lemey, P., & Van Ranst, M. (2010). Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. *Molecular Biology and Evolution*, 27, 2431-2436. <https://doi.org/10.1093/molbev/msq137>
- Maunula, L., & von Bonsdorff, C. H. (2002). Frequent reassortments may explain the genetic heterogeneity of rotaviruses: analysis of Finnish rotavirus strains. *Journal of Virology*, 76, 11793-11800. DOI: 10.1128/JVI.76.23.11793-11800.2002

- Mohamed, F. F., Mansour, S. M., El-Araby, I. E., Mor, S. K., & Goyal, S. M. (2017). Molecular detection of enteric viruses from diarrheic calves in Egypt. *Archives of Virology*, 162, 129-137. <https://doi.org/10.1007/s00705-016-3088-0>
- Moussa, A., Fredj, M. B., BenHamida-Rebai, M., Fodha, I., Boujaafar, N., & Trabelsi, A. (2017). Phylogenetic analysis of partial VP7 gene of the emerging human group A rotavirus G12 strains circulating in Tunisia. *Journal of Medical Microbiology*, 66, 112-118. [10.1099/jmm.0.000420](https://doi.org/10.1099/jmm.0.000420)
- Oberste, M. S., Feeroz, M. M., Maher, K., Nix, W. A., Engel, G. A., Hasan, K. M., Begum, S., Oh, G., Chowdhury, A. H., & Pallansch, M. A. (2013). Characterizing the picornavirus landscape among synanthropic nonhuman primates in Bangladesh, 2007–2008. *Journal of Virology*, 87, 558-571. DOI: [10.1128/JVI.00837-12](https://doi.org/10.1128/JVI.00837-12)
- Payne, D. C., Edwards, K. M., Bowen, M. D., Keckley, E., Peters, J., Esona, M. D., Teel, E. N., Kent, D., Parashar, U. D., & Gentsch, J. R. (2010). Sibling transmission of vaccine-derived rotavirus (RotaTeq) associated with rotavirus gastroenteritis. *Pediatrics*, 125, e438-e441.
- PREDICT One Health Consortium 2016. PREDICT Operating Procedures: Non-Human primate sampling.
- Rahman, M., Sultana, R., Ahmed, G., Nahar, S., Hassan, Z. M., Saiada, F., Podder, G., Faruque, A. S., Siddique, A. K., Sack, D. A., Matthijnssens, J., Ranst, M. V., & Azim. T. (2007). Prevalence of G2P [4] and G12P [6] rotavirus, Bangladesh. *Emerging Infectious Diseases*, 13, 18. <https://dx.doi.org/10.3201%2F1301.060910>
- Rajendran, P., & Kang, G. (2014). Molecular epidemiology of rotavirus in children and animals and characterization of an unusual G10P[15] strain associated with bovine diarrhea in south India. *Vaccine*, 32, A89-A94. <https://doi.org/10.1016/j.vaccine.2014.03.026>
- Rotavirus classification working group - RCWG. Newly assigned genotypes-Update May 29 2018. Retrieved from <https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg> (accessed 23 October 2019)

- Richard, A. F., Goldstein, S. J., & Dewar, R. (1989). Weed macaques: the evolutionary implications of macaque feeding ecology. *International Journal of Primatology*, 10, 569-594. <https://doi.org/10.1007/BF02739365>
- Sai, L., Sun, J., Shao, L., Chen, S., Liu, H., & Ma, L. (2013). Epidemiology and clinical features of rotavirus and norovirus infection among children in Ji'nan, China. *Virology Journal*, 10, 302. doi:10.1186/1743-422X-10-302
- Santos, N., & Hoshino, Y. (2005). Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Reviews in Medical Virology*, 15, 29-56. <https://doi.org/10.1002/rmv.448>
- Saudy, N., Elshabrawy, W. O., Megahed, A., Foad, M. F., & Mohamed, A. (2016). Genotyping and Clinicoepidemiological Characterization of Rotavirus Acute Gastroenteritis in Egyptian Children. *Polish Journal of Microbiology*, 65, 433-442.
- Schillaci, M. A., Jones-Engel, L., Engel, G. A., Paramastri, Y., Iskandar, E., Wilson, B., Allan, J. S., Kyes, R. C., Watanabe, R., & Grant, R. (2005). Prevalence of enzootic simian viruses among urban performance monkeys in Indonesia. *Tropical Medicine & International Health*, 10, 1305-1314. <https://doi.org/10.1111/j.1365-3156.2005.01524.x>
- Sha, J. C. M., Gumert, M. D., Lee, B. P. H., Jones-Engel, L., Chan, S., & Fuentes, A. (2009). Macaque-human interactions and the societal perceptions of macaques in Singapore. *American Journal of Primatology*, 71, 825-839. <https://doi.org/10.1002/ajp.20710>
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729. <https://doi.org/10.1093/molbev/mst197>
- Tate, J. E., Burton, A. H., Boschi-Pinto, C., Steele, A. D., Duque, J., & Parashar, U. D. (2012). 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. *The Lancet Infectious Diseases*, 12, 136-141. doi: 10.1016/S1473-3099(11)70253-5
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by



quality analysis tools. *Nucleic Acids Research*, 25, 4876-4882.  
<https://doi.org/10.1093/nar/25.24.4876>

Tort, L. F. L., Victoria, M., Lizasoain, A., García, M., Berois, M., Cristina, J., Leite, J. P. G., Gómez, M. M., Miagostovich, M. P., & Colina, R. (2015). Detection of common, emerging and uncommon VP4, and VP7 human group A rotavirus genotypes from urban sewage samples in Uruguay. *Food and environmental virology*, 7, 342-353. <https://doi.org/10.1007/s12560-015-9213-5>

Wylie, K. M., Stanley, K. M., Tekippe, E. M., Mihindukulasuriya, K., & Storch, G. A. (2017). Resurgence of rotavirus genotype G12 in St. Louis during the 2014-2015 rotavirus season. *Journal of the pediatric infectious diseases society*, 6, 346-351. doi: 10.1093/jpids/piw065

Xue, C., Raveendran, M., Harris R. A., Fawcett, G. L., Liu, Xiaoming., White, S., Dahdouli, M., Deiros, D. R., Below, J. E., Salerno, W., Cox, L., Fan, Guoping., Ferguson, B., Horvath, J., Johnson, Z., Kanthaswamy, S., Kubisch, M. H., Liu, D., Platt, M., Smith, D. G., Sun, B., Vallender, E. J., Wang, F., Wiseman, R. W., Chen, R., Muzny, D. M., Gibbs, R. A., Yu, F., & Rogers, J. (2016) The population genomics of rhesus macaques (*Macaca mulatta*) based on whole-genome sequences. *Genome Research*, 26, 1651-1662. <https://dx.doi.org/10.1101%2Fgr.204255.116>

**Table 1: Distribution of rotavirus in *M. mulatta* by age, sex and habitat in Bangladesh, 2013 (N=454)**

Variables	Category	Number Positive (%)	95% CI*
Age	Adult (266)	13(4.9)	2.63-8.21
	Neonate (47)	2(4.3)	0.52-14.54

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	Sub-adult (141)	5(3.6)	1.16-8.08
Sex	Female (234)	9(3.85)	1.77-7.18
	Male (220)	11(5.0)	2.52-8.77
Types of habitat	Peri-urban (131)	7(5.34)	2.18-10.7
	Rural (122)	5(4.1)	1.34-9.31
	Urban (201)	8(3.98)	1.73-7.69

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\*CI-Confidence Interval

**Table 2: Genotypes of RVA strains found in rhesus macaques (*M. mulatta*) by site in Bangladesh, 2013**

No.	Genotype	Isolate ID	Sampling Area
1	G3	PGB-690	Bandar, Narayanganj
2	G3	PGB-691	Bandar, Narayanganj
3	G3P[3]	PGB-692	Bandar, Narayanganj
4	G3	PGB-700	Bandar, Narayanganj
5	G3	PGB-701	Bandar, Narayanganj
6	G10 P[15]	PGB-876	Rampur, Narsingdi
7	G3 P[15]	PGB-980	Charmuguria, Madaripur

**Figure legends:**

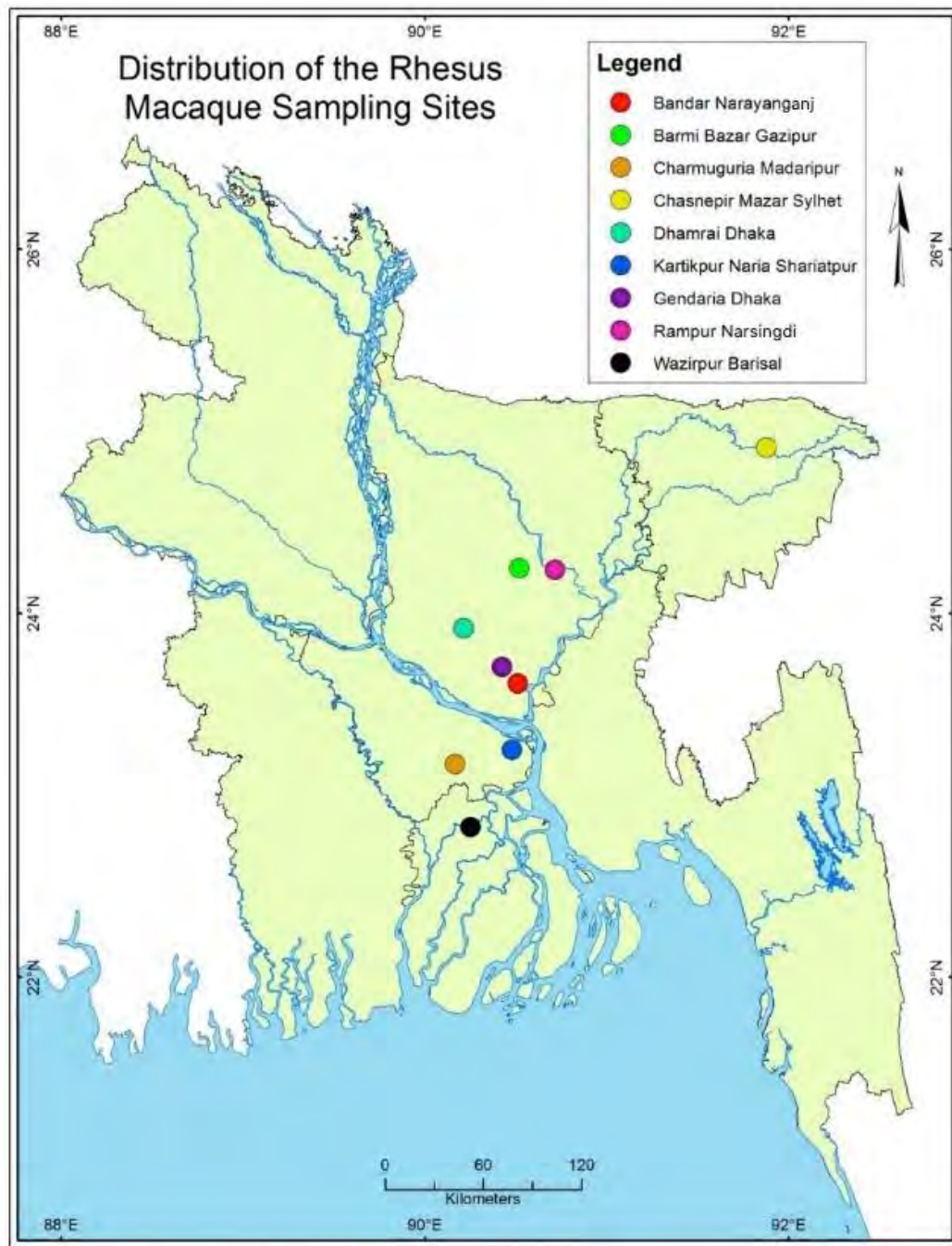
**Figure 1:** Rhesus macaque sampling sites (9 areas) from Bangladesh, 2013

**Figure 2:** Phylogenetic analysis of nucleotide sequence of VP7 gene of G3 rotavirus strains from Bangladesh 2013. Reference sequences were retrieved from the GenBank database. The study strains were denoted with black triangle (▲). Boot-strap values (1000 pseudo-replicates) above 80 are shown. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [1]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 27 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 835 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [2].

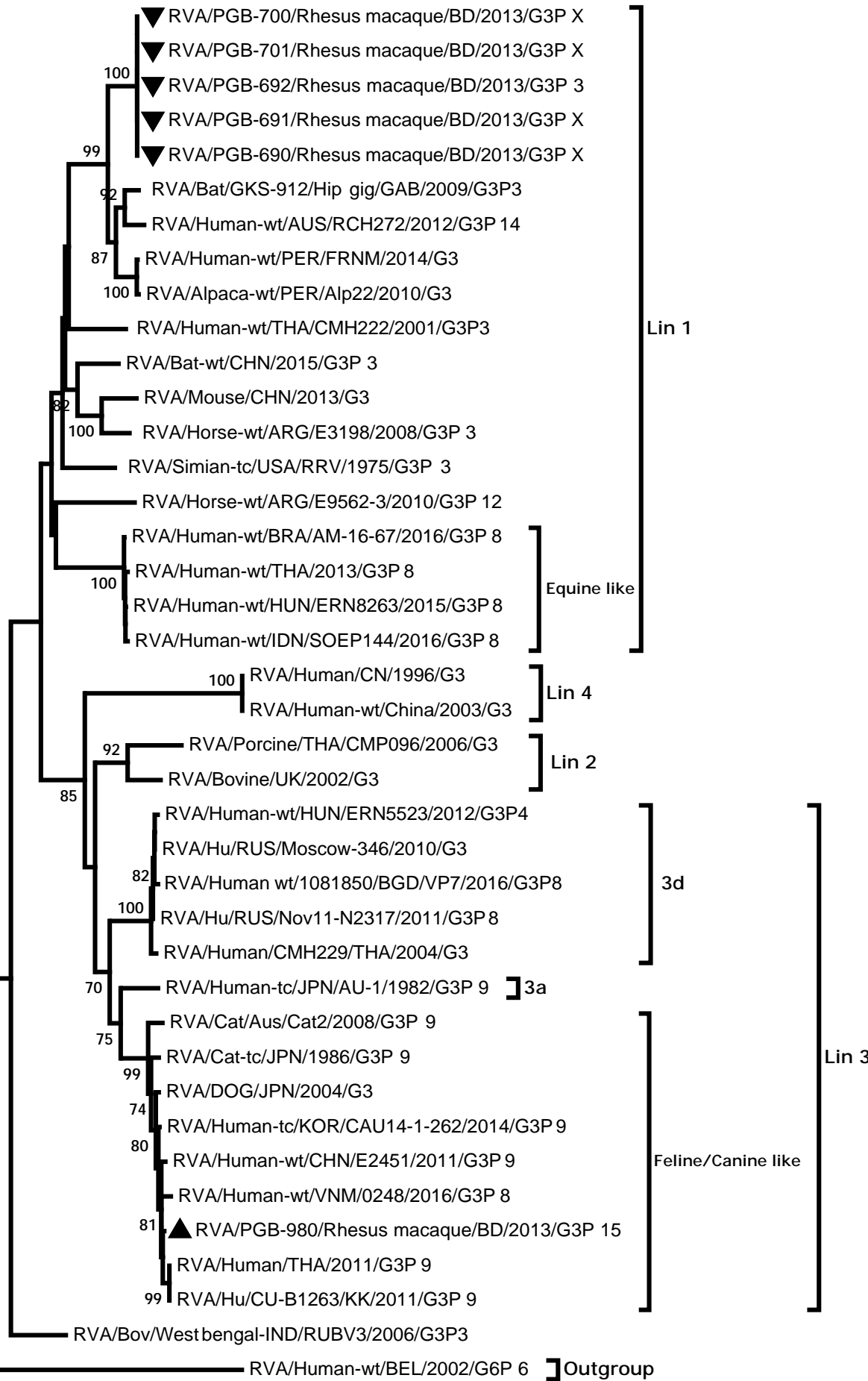
**Figure 3:** Phylogenetic analysis of nucleotide sequence of VP7 gene of G10 rotavirus strains from Bangladesh 2013. Reference sequences were retrieved from the GenBank database.

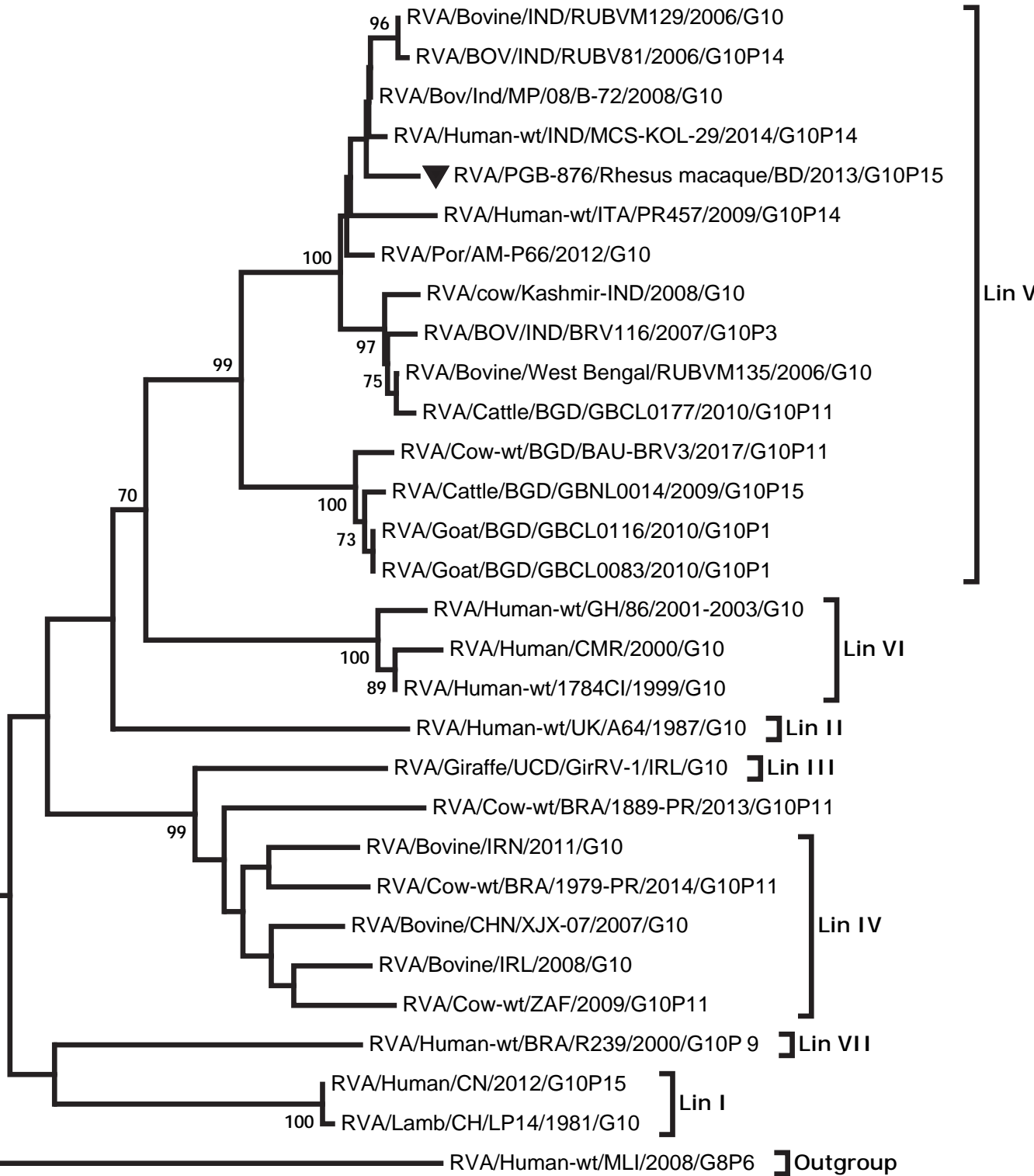
**Figure 4:** Phylogenetic analysis of nucleotide sequence of VP4 gene of P[15] rotavirus strains from Bangladesh 2013. Reference sequences were retrieved from the GenBank database.

**Figure 5:** Phylogenetic analysis of nucleotide sequence of VP4 gene of P[3] rotavirus strains from Bangladesh 2013. Reference sequences were retrieved from the GenBank database.

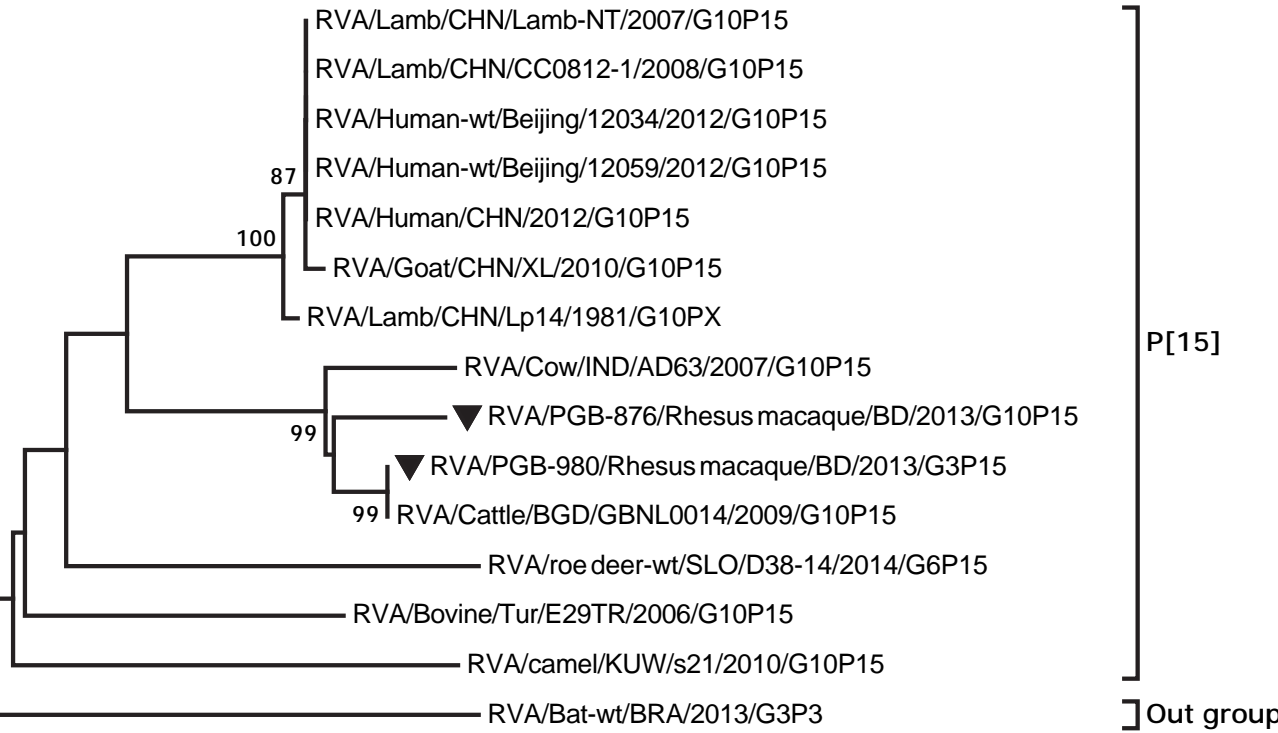


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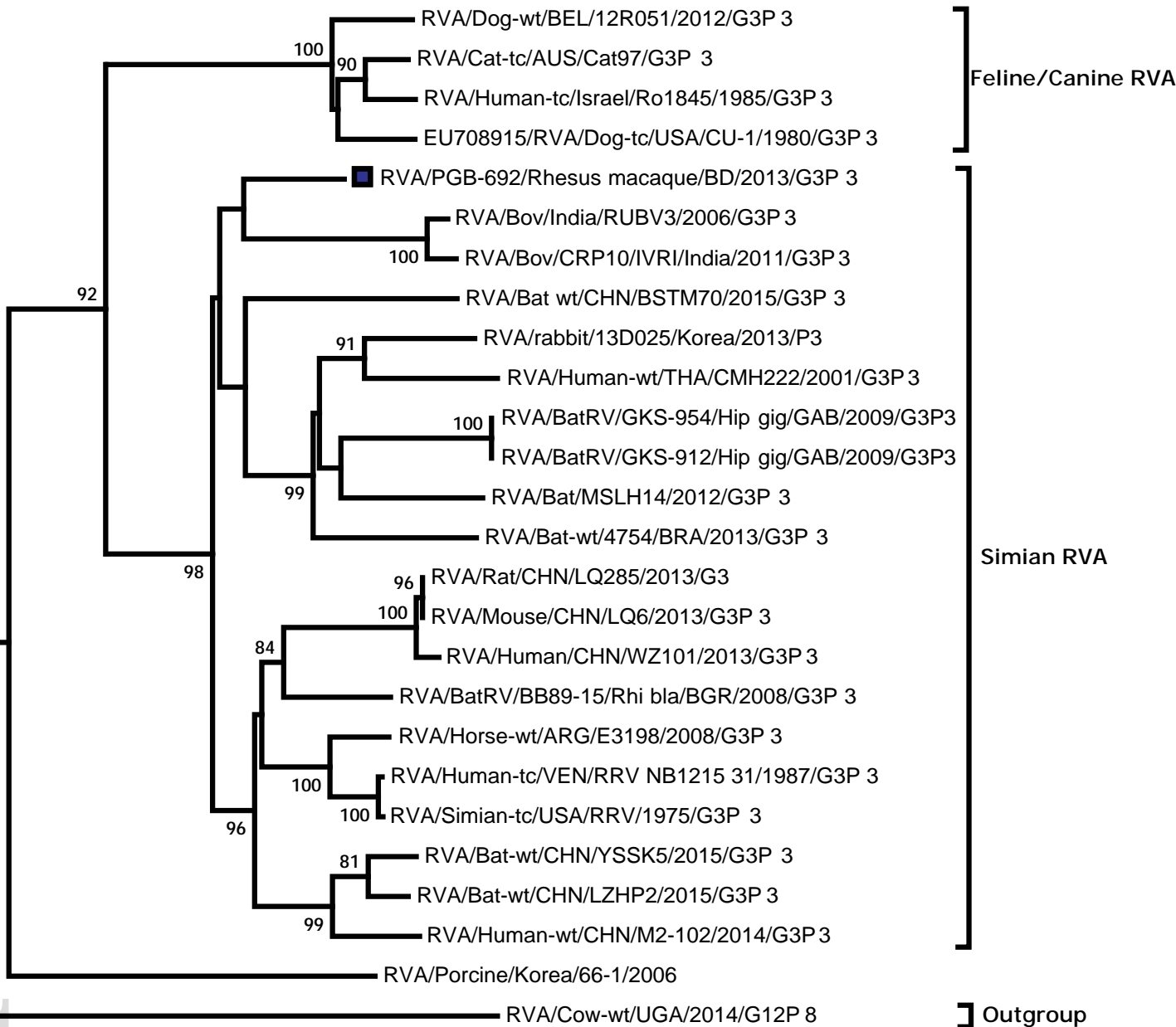




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