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Original Article

Epidemiology and genotypes of group A rotaviruses in cattle and goats of Bangladesh, 2009-2010

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Abstract:

Group A rotavirus (RVA) is recognized as a major cause of severe gastroenteritis in newborn calves and goat kids. We estimated the proportion of ruminants infected with rotavirus and identified the circulating genotypes in cattle and goats in Bangladesh. Between May 2009 and August 2010, fecal samples were collected from 520 cattle and goats presenting with diarrhea at three government veterinary hospitals in three districts of Bangladesh. All samples were screened for RVA RNA using real-time, one-step, reverse transcription polymerase chain reaction (qRT-PCR). Of the 520 animals tested, 11.7% (61) were positive for RVA RNA, with 6.2% (15/241) and 16.5% (46/279) positivity in cattle and goats, respectively. RVA positive samples were further characterized by nucleotide sequence analysis of two structural protein gene fragments, VP7 (G genotype), and VP4 (P genotype). Among 17 successfully sequenced strains, G8 (17.9%) was the most prevalent G-genotype followed by G10 (8%) and G6 (1.6%). P[1] (11.3%) was the most frequently detected P-genotype followed by P[11] (3.2%) and P[15] (1.6%). The most common VP7/VP4 combinations for cattle were G10P[11], G10P[15], and G6P[11], and for goat, G8P[1], and G10P[1]. Phylogenetic analysis of the RVA strains showed clustering with bovine and caprine strains from neighboring India. The study adds to our understanding of the genetic diversity of bovine and caprine rotavirus strains in Bangladesh. Our findings highlight the importance of rotavirus surveillance in cattle and goat populations, which may serve as a potential source for genetic reassortment and zoonotic transmission.

Keywords: Rotaviruses, bovine, caprine, rotavirus genotypes, Bangladesh

1. Introduction

Group A rotavirus (RVA) is the leading cause of neonatal calf diarrhea and livestock mortality worldwide. RVA infects multiple animal species including young calves, hogs, foals and goats, which incurs substantial economic losses (Reinhardtet al.,1986; Dhama et al., 2009). A number of RVA strains are zoonotic and contribute to rotavirus infection as the leading cause of diarrhea-associated morbidity and mortality globally among children under the age of 5 years (Troeger et al., 2018). In Bangladesh, approximately 2700 children younger than 5 years die each year due to RVA diarrhea (Pecenka et al., 2017).

RVA belongs to the *Reoviridae* family, which is comprised of ten genetically distinct groups (A-J) (Banyai et al., 2017). The RVA genome is comprised of 11 double-stranded RNA (dsRNA) segments, which encodes six structural proteins and five or six non-structural proteins (Estes & Kapikian, 2007). The genome is encapsulated by a triple-layered capsid. Each layer of the capsid is composed of distinct viral proteins, with the innermost layer composed of VP2 proteins, VP6 in the middle layer and the outermost layer composed of VP4 and VP7 proteins. The outermost VP4 and VP7 viral capsid proteins determine the P genotype and G genotype of the virus (Matthijnssens et al., 2010).

Agriculture and livestock are key components of the Bangladesh economy. Approximately 24 million cattle and 26 million goats are currently reared in the country, with more than 62% of households raising livestock for household income (DLS, 2019; Rabbani et al., 2004). Calf diarrhea is one of the leading health complications reported in both the dairy industry and rural cattle and goat farming in Bangladesh, which results in substantial economic losses (Debnath et al., 1990).

Studies of RVA in cattle and goats in Bangladesh have been limited to serological studies describing the presence of RVA antibodies. Previous reports observed between 7% - 22% RVA infection in calves (Alam et al., 1999; Selim et al., 1991), while studies of RVA in Black Bengal goats reported 8.68% prevalence of infection (Dey et al., 2007). Other studies

in Bangladesh have reported on the presence of RVA in poultry farm broiler bird, with 13.15% of birds infected with RVA(Deyet al., 2007; Islam et al.,2009). However, there are no previous studies that have combined epidemiological, clinical and genetic data sources to characterise the patterns of RVA infection in cattle and goats in Bangladesh.

Unlike in domestic animals, the epidemiology and genetic diversity of human RVA in Bangladesh has received greater focus (Haque et al., 2018; Rahman et al., 2011; Zaman et al., 2009). In 2011, a rare bovine-like human VP4 mono-reassortant G6P[8] RVA was detected in an infant during an ongoing diarrhea etiology surveillance in Mirzapur, Bangladesh (Afrad et al., 2013). Phylogenetic analysis of all 11 genes suggested that the strain was a mono-reassortant between a human RVA strain and a bovine-like strain, most likely resulting from a direct zoonotic transmission event of the bovine-like RVA strain, followed by the reassortment event. Interspecies transmission events such as this have been shown to more common in developing countries, where the close proximity between human and livestock populations increases the likelihood of interspecies transmission or emergence of novel zoonotic RVA. Therefore, the examination of domestic livestock as a potential source for human RVA infection is warranted.

In this study, we present data on the frequency and genetic diversity of RVA circulating in cattle and goats of Bangladesh. We present a characterization of the genetic diversity of RVA before conducting phylogenetic comparisons against previously reported RVA strains. These findings further our understanding of the circulating diversity of RVA in livestock in Bangladesh, which has implications for reducing livestock mortality, economic losses, as well as the introduction of a RVA vaccine in livestock of Bangladesh.

2. Materials and Methods

2.1*Enrollment of ruminants and collection of samples*

Between May 2009 and August 2010, we carried out veterinary hospital-based surveillance to identify the emerging and reemerging zoonotic diseases in cattle and goats in three districts of Bangladesh: Netrokona in the eastern region, Dinajpur, in the northern region, and Chattogram in the south. The veterinary surgeon (VS) at each hospital enrolled cattle and goats presenting with diarrhea and/or other clinical symptoms. The VS interviewed the animal owners and recorded the demographic information of the animals, which included age, breed, date of onset of illness, clinical signs/symptoms and nutritional status of ruminants. The team collected two fecal swabs in lysis buffer from each enrolled animal maintaining aseptic measures. The samples were preserved in a dry shipper maintaining temperature -150°C or below until tested at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) virology laboratory.

We conducted follow-up visits to the households of each animal two weeks after enrollment to obtain the clinical outcomes of the infection. The date of onset was categorized into two categories depending on the monthly average temperature. We classified warm months as those with mean temperatures >=25^0C and cold months as those with mean temperatures below 25^0C using data from the Bangladesh Meteorological department (BMD, 2019). Livestock were also categorized in age categories. We considered cattle less than 12 months old as calves and goats less than 6 months as juvenile goats/kids.

2.2 *Ethics statement*

This study protocol (icddr,b; protocol 2008-074) was reviewed and approved by the research review committee, ethical review committee and animal experimentation ethics

committee of iccdr,b. Informed written consent was obtained from the owners of the cattle and goats prior to enrollment.

2.3 *RNA extraction and polymerase chain reaction*

Viral RNA was extracted from the fecal samples using 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- transcription polymerase chain reaction (qRT-PCR) according to the procedure as described previously (Jothikumar et al., 2009).

To identify the G and P genotype, conventional reverse-transcription polymerase chain reaction (RT-PCR) was performed to amplify the VP7 and VP4 genes fragments using consensus primer pairs VP7F/VP7R (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, as described elsewhere (Gentsch et al., 1992; Pang et al., 1999; Rahman et al., 2007). RT-PCR was carried out using the QIAGEN® One-step RT-PCR Kit (QIAGEN, Germany) according to the manufacturer's instructions.

Denaturation of viral dsRNA was carried out by heating at 95°C for 5 minutes and subsequent rapid cooling on ice before the actual reverse transcription reaction. After that the RT-PCR reaction was carried out with an initial reverse transcription step at 45°C for 30 min, followed by PCR activation at 95°C for 15 min, 35 cycles of amplification (30 s at 94°C, 30 s at 48°C, 60 s at 72°C) and a final extension at 72°C for 7 min in a thermal cycler (T3000 Thermocycler, Biometra, USA). The amplified PCR products were analyzed by 1.5% agarose gel, stained with ethidium bromide and visualized under a UV transilluminator (Bio-Rad Laboratories, CA, USA).

2.4 *Nucleotide Sequencing and phylogenetic analysis*

Nucleotide sequencing of amplified PCR products was carried out in an automated ABI3500 xL Genetic Analyzer (Applied Biosystem, Foster City, CA, USA) and Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem) as per kit protocol. The chromatogram files were inspected using Chromas 2.23 (Technelysium, Helensvale, Australia). The genotypes of the sequences were confirmed using the rotavirus genotyping tool 'RotaC$^{2.0}$'(Maeset al., 2009). All rotavirus VP7 and VP4 gene sequences generated in this study were submitted to the GenBank database under the accession numbers MK519588 through MK519614.

Sequence similarity searches were performed using the Basic Local Alignment Search Tool (BLAST) server on the GenBank database to retrieve previously collected sequences with high similarity. Sequences were aligned by ClustalW algorithm using BioEdit (version 7.0.9.0). Maximum likelihood phylogenetic tree with 1000 bootstrap method was constructed by using MEGA-X (version 10.0.5) after finding the best nucleotide substitution model in 'Find best DNA/ Protein Models (ML)' program for each viral gene. The final phylogenetic tree was graphically presented with FigTree (version 1.4.4) software.

2.5 *Statistical analysis*

The proportion of each laboratory-confirmed rotavirus infection associated with age, sex, breed, geographical locations, and the months of sampling was analyzed through chi-square or Fisher's exact test where appropriate in the bivariate analysis. We calculated the odds ratio with a 95% confidence interval (CI) using logistic regression as our outcome variable was dichotomous. We performed multivariate logistic regression for both cattle and goats separately to adjust confounding variables to estimate the adjusted odds ratio (aOR) and 95% CI by selecting variables that remain significant ($p < 0.05$) in bivariate analysis. To assess for

a correlation between sampling time and RVA positivity, linear regression was used and the correlation coefficient reported.

3. Results

3.1 Epidemiology of rotaviruses in ruminants

Of the 520 animal samples, 46.3% (241/520) were from cattle and 53.7% (279/520) were from goats (Table 1). The detectable RNA for rotavirus was found in11.7% (61/520) ruminants: 6.2 % (15/241) in cattle and 16.5% (46/279) in goat. In bivariate analysis, the proportion of rotavirus-positive samples varied according to their species, age, and months of sampling. The median age of PCR-positive cattle was 24 (range: 8-42) months and for the goat was 12 months (range: 6-24). Rotavirus A RNA was more frequently detected in younger goats (30%, 95% CI: 15-45%) than in older goats (14.2%, 95% CI: 10-19%).

A number of other symptoms were present in RVA-positive ruminants, including fever (88.5%, n= 54), nasal discharge (36.1%, n=22), respiratory distress (32.8%, n=20), and coughing (27.9%, n=17) (Table 2). During the hospital visit of the animals, 91.8% of RVA-positive animals received antimicrobial treatment without any laboratory-confirmed bacterial etiology. Follow-up visits indicated that 54.1% (33/61) of animals recovered from the infection, nevertheless, 14.8% (9/61) died, 14.8% (9/61) remained sick, and 11.5% (7/61) were sold (Table 2).

We observed a positive correlation between average temperature of the months of sampling and RVA positivity (correlation coefficient: 0.168), the prevalence of RVA was associated with increased environmental temperature among the enrolled animals (Figure 1). Compared to the cold months, RVA were more frequently detected in warm months for goats (aOR: 4.09, CI: 1.41-11.95) (Table 3), however, no significant differences observed in cattle.

3.2 *Distribution of G and P genotypes*

Genotype G8 was dominant followed by G10, and G6, with various combinations of both G and P types, detected. In our study, the three G genotypes (G6, G8, and G10) were combined mostly with three P genotypes (P[1], P[11] and P[15]). Overall, G8P[1] (8.1%) was the predominant strain, followed by G10P[1] (3.2%), G10P[15] (1.6%), G10P[11] (1.6%), and G6P[11] (1.6%). RVA co-infection was not detected in any of the samples. Because only traditional RT-PCR was performed, assessment for mixed genotypes using multiplex PCR was not done.

3.3 *Sequence analysis*

A total of 17 gene sequences at least 700-bp in length were found to contain the VP7 gene, and 10 gene sequences at least 600-bp in length contained the VP4 gene. BLAST searches against each gene were used to identify additional VP7 and VP4 sequences respectively from GenBank. These sequences were used initially to confirm pairwise identity frequency graphs on the nucleotide level as well as for the following phylogenetic analysis. The identity graphs yielded suitable identity cut-offs equal to 80% for both VP7 and VP4. Only GBCL0083 and GBCL0116 strains had an intra-genotype identity less than 80% (both sequences had a 79.2% shared identity with South African isolate RVA/Cow-wt/ZAF/JN831223/2007/G8P[1]). In addition, inter-genotype identities for VP4 were contained with two clear ranges, with comparisons between P[15] and P[1] having identities between 67.9% - 71.9% and comparisons involving P[11] have identities between 49.0% - 52.5%.

3.4 *Phylogenetic analysis of VP7 and VP4 Gene*

Phylogenetic analyses were performed using the Tamura-3 parameter and gamma distributed with invariant sites (T92+G+I) model, which was found to be the most suitable

from model selection for the VP7 gene (Supplementary file: S1). All of our study strains clustered with rotavirus strains isolated from India, most of which were from bovine origin (Figure 2). All five strains of G10 genotype clustered in Clade-1 with circulating strains in India and Bangladesh as well as two human strains from Japan (Mc35) and Italy (PR457). Three strains GBCL0116, GBCL0083 and GBNL0014 clustered in the same sub-cluster (Clade-1.c), while strains GBCL0193 and GBCL0177clustered in two different sub-clusters - Clade-1.b and Clade-1.a, respectively. Both Clade-1.a and Clade-1.c contained only bovine strains, but Clade-1.b was comprised of caprine, bovine, and human strains. Among the 11 strains with G8 genotypes, 8 (GBHL0425, GBHL0397, GBHL0387, GBHL0414, GBCL0211, GBNL0022, GBHL0377, and GBHL0376) strains clustered within the same clade (Clade-3.b). Two caprine strains GBCL0286 and GBCL0200 clustered with Indian bovine and caprine strains (Clade-3.a), demonstrating that Clade-3was circulating within bovine and caprine populations in India and Bangladesh. A third caprine strain GBHL0353 clustered with an Indian human rotavirus strain in a different sub-cluster. The only G6 strain GBHL0381in our study clustered with Clade-2 and showed 100% similarity to other bovine strains from India and Bangladesh.

The Tamura-3 parameter and gamma distribution (T92+G) model was also identified as the most suitable for phylogenetic analyses of the VP4 gene for our 10 strains via model selection (Supplementary file: S1). Similar to VP7, the strains of VP4 gene also clustered with Indian and Bangladeshi bovine rotavirus strains (Figure 3). All of our strains of P[1] genotype clustered in Clade-1 together with other strains circulating within South and Southeast Asia. Five strains (GBHL0397, GBHL0387, GBNL0022, GBHL0376, and GBCL0211) out of seven P[1] genotype clustered together in Clade-1.b with other Bangladeshi caprine strains identified in 1999; while two other strains (GBCL0116 and GBCL0083) clustered in another clade with Indian bovine strains (Clade-1.a). Our two strains

(GBHL0381 and GBCL0177) of P[11] genotype clustered with an Indian bovine strain in Clade-3. Our only strain of P[15] genotype (GBNL0014) stands clearly separated from other P[15] bovine strains, however, clustered with a Bangladeshi strain isolated from *Rhesus macaque* in Clade 2.

4. Discussion

We found overall 11.7% ruminants presenting with diarrhoea visiting veterinary hospitals in Bangladesh were infected with RVA. The proportion of animals confirmed with RVA in cattle was similar to a previous report, which reported a 7% prevalence in calves less than one year of age (Selim et al., 1991). However, it was also lower than a more recent study, which reported 22% prevalence in diarrheic calves from Bangladesh (22%) (Alam et al., 1999). The potential reasons for this variation might have been influenced by diagnostic procedure, age differences, sampling strategy, and clinical status of the study population. Several reports from India demonstrate a higher prevalence than in our study (11.8-27 %) (Jindal et al., 2000; Beget al., 2010; Dashet al., 2011;), while reports from Pakistan demonstrated lower prevalence (3%) (Mukhtar et al., 2017).

The prevalenceof RVA observed in the goat populationn was also higher than a previous report (8.7%) from Bangladesh where diarrheic juvenile kids were examined by RNA polyacrylamide-gel-electrophoresis and silver staining (PAGE-ss) in 2007 (Dey et al., 2007). This difference may be due to differences in diagnostic sensitivity as well as spatio-temporal variability between the samples and study populations. Outside of Bangladesh, there are few epidemiologic reports of RVA in goats from South Asia. Two recent reports from India, however, report between eight and fifteen percent RVA prevalence in caprine samples across three different states of India (Reddy et al., 2014; Singh et al., 2017). High RVA prevalence in cattle and goats may demand the continuous monitoring of RVA through routine

surveillance, as well warranting the introduction of a rotavirus vaccination program in domestic livestock of Bangladesh. Rotavirus vaccination has proven effective in the control of calf diarrhea in beef herds of Argentina (Bellinzoni et al., 1989).

Our study included samples from three geographically distant regions in Bangladesh. We found a higher frequency of rotavirus in ruminants during the warm months (summer season), which differs with recent findings by Barua et al., where the winter season was found to have higher odds of having the RVA infections (Barua et al., 2019). In their study, RVA infections in neonatal calves (<6 weeks) originating from Chattogram were more prevalent in the winter. The differences in prevalence may be either due to our sample scheme only spanning a one and half-year period or because we sampled both young and adult animals, which was shown to be a significant risk factor of RVA infection. However, whereas human rotavirus prevalence peaks in winter in temperate regions (Glass et al., 1996), there are less obvious seasonal patterns in tropical countries such as Bangladesh (Alam et al., 2011; Stoll et al., 1982).

The predominant genotypes circulating in our samples were G8 followed by G10 in combination with P[1] and P[11]. In particular, all samples collected from bovine sources contained P[11] and P[15], which is in agreement with other studies identifying G10P[11] as a frequently detected strain in dairy livestock (Badaracco et al., 2013). BLAST searches revealed our G8 strains shared more than 95% pairwise sequence identity with human G8 strains. However, similarity search results of the G8 strain provide evidence of human-bovine reassortment of rotavirus VP7 gene. In phylogenetic analysis with contemporary strains, our strains were clustered with bovine strains isolated from India, which is presumed to be driven by the large-scale cattle trade between India and Bangladesh. As a result, future iterations of this study should record the travel information of the livestock and their owners, which may

provide more insight into the population structure and whether additional advice should be given to livestock owners regarding quarantine.

This study has a few limitations which should be considered when interpreting the findings. First, we carried out the study in veterinary hospital and did not include community-level diarrheic cattle and goats. In Bangladesh, only a small proportion of farmers bring their sick animals to the veterinary hospital, as many cannot afford the travel cost (Rahman et al., 2018). Second, we only enrolled diarrheic cattle and goats; therefore, we may have missed asymptomatic rotavirus infections (Archambault et al., 1990). Third, we were not able to retrieve the nucleotide sequence data from all the PCR-positive clinical samples. As such we may fail to capture the complete genetic diversity of ruminant rotaviruses in Bangladesh. In addition, our samples were collected during 2009-10 and are thus only representative of the circulating strains and seasonal patterns for that period due to likely recent changes through genetic reassortment (McDonald et al., 2016). Lastly, our genotyping analysis was based on partial sequences of VP7 and VP4 genes and did not cover the full genome constellation of circulating rotavirus strains in cattle and goat population of the country.

Despite the limitations, our study provides epidemiological and genotypic information on rotavirus in cattle and goats. The study highlights the importance of bovine and caprine rotavirus surveillance in Bangladesh due to the potential for viral sharing between domestic animals and humans (Cook et al., 2004). We have submitted twenty one caprine and six bovine genome sequences in the GenBank from this study, which will remain as a valuable resource for understanding the evolution of rotaviruses in ruminants and future rotavirus research in Bangladesh and South Asia. While our study helps to understand the rotavirus diversity in cattle and goats of Bangladesh, tissue culture-based rotavirus isolation followed by whole-genome sequencing is needed to fully understand strain diversity and the potential for antigenic reassortment.

Author Contributions: MBH, MSR, and NH conceived and coordinated the study and wrote the paper. OJW, SR, MAHK contributed to data analysis, interpretation of the results. MSR, RH performed laboratory procedures, including diagnostic testing, sequencing and genotyping. AI, MGO, JHE, PD, and NH reviewed the article critically for intellectual content. All authors reviewed subsequent drafts of the manuscript and approved the final version.

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Table 1

Demographic characteristics of cattle and goats enrolled at three veterinary hospitals in Bangladesh during May 2009 and August 2010

Variables		**Number of samples tested**	**RVA positive n (%)**
Sample Origin (Districts)	Netrokona	96	5 (5.2)
	Dinajpur	306	28 (9.2)
	Chattogram	118	28 (23.7)
Species	Cattle	241	15 (6.2)
	Goat	279	46 (16.5)
	Overall	520	61 (11.7)
Cattle Breed	Local breed	217	12 (5.5)
	Cross-bred	24	3 (12.5)
Goat Breed	Cross-bred	21	4 (19.0)
	Black Bengal	212	31 (14.6)
	Jamunapari	46	11 (23.9)
Sex of the animal	Male	181	19 (10.5)
	Female	339	42 (12.4)
Months of onset of illness	Cold months(<=25^{0}C)	143	5 (3.5)
	Warm months (>25^{0}C)	377	56 (14.9)

Table 2

Clinical signs and outcome of the rotavirus A (RVA) positive animals enrolled at three veterinary hospitals in Bangladesh during May 2009 and August 2010

Variables	Clinical signs/outcomes	Frequency	Percent
Clinical signs	Diarrhoea	61	100
	Fever	54	88.5
	Nasal discharge	22	36.1
	Respiratory distress	20	32.8
	Coughing	17	27.9
Outcome of RVA-positive animals (n=61) at 14 days	Recovered	33	54.1
	Death	9	14.8
	Remained sick	9	14.8
	Sold	7	11.5
	Slaughtered	1	1.6
	Lost to follow-up	2	3.3

Table 3

The adjusted odds ratios (aOR) of rotavirus A (RVA) infections among cattle and goats presenting with compatible clinical signs at three government veterinary hospitals in Bangladesh between May 2009 and August 2010

Variables	No. of samples tested	RVA positive n (%)	Bivariate analysis OR (95% CI)	Multivariate analysis aOR (95% CI)
Species				
Cattle	241	15 (6.2)	Ref.	-
Goat	279	46 (16.5)	2.97 (1.61-5.48)	-
Cattle Age				
Adults	194	11 (5.7)	Ref.	-
Calves (<12 months)	47	4 (8.5)	1.55 (0.47-5.09)	-
Goat Age				
Adult	239	34 (14.2)	Ref.	Ref.
Kids (<6 months)	40	12 (30)	2.58 (1.20-5.57)	2.52 (1.09- 5.87) *
By breed (Cattle)				
Local	217	12 (5.5)	Ref.	-
Cross	24	3 (12.5)	2.44 (0.64-9.34)	-
By breed (Goat)				
Black Bengal	212	31 (14.6)	Ref.	-
Cross	21	4 (19.0)	1.37(0.43-4.35)	-
Jamunapari	46	11 (23.9)	1.83 (0.84-3.99)	-
Location (Cattle)				
Netrokona	81	4 (4.9)	Ref.	-
Dinajpur	150	9 (6))	1.22 (0.37-4.12)	-
Chattogram	10	2 (20)	4.81 (0.76-30.51)	-
Location (Goat)				
Netrokona	15	1 (6.7)	Ref.	-

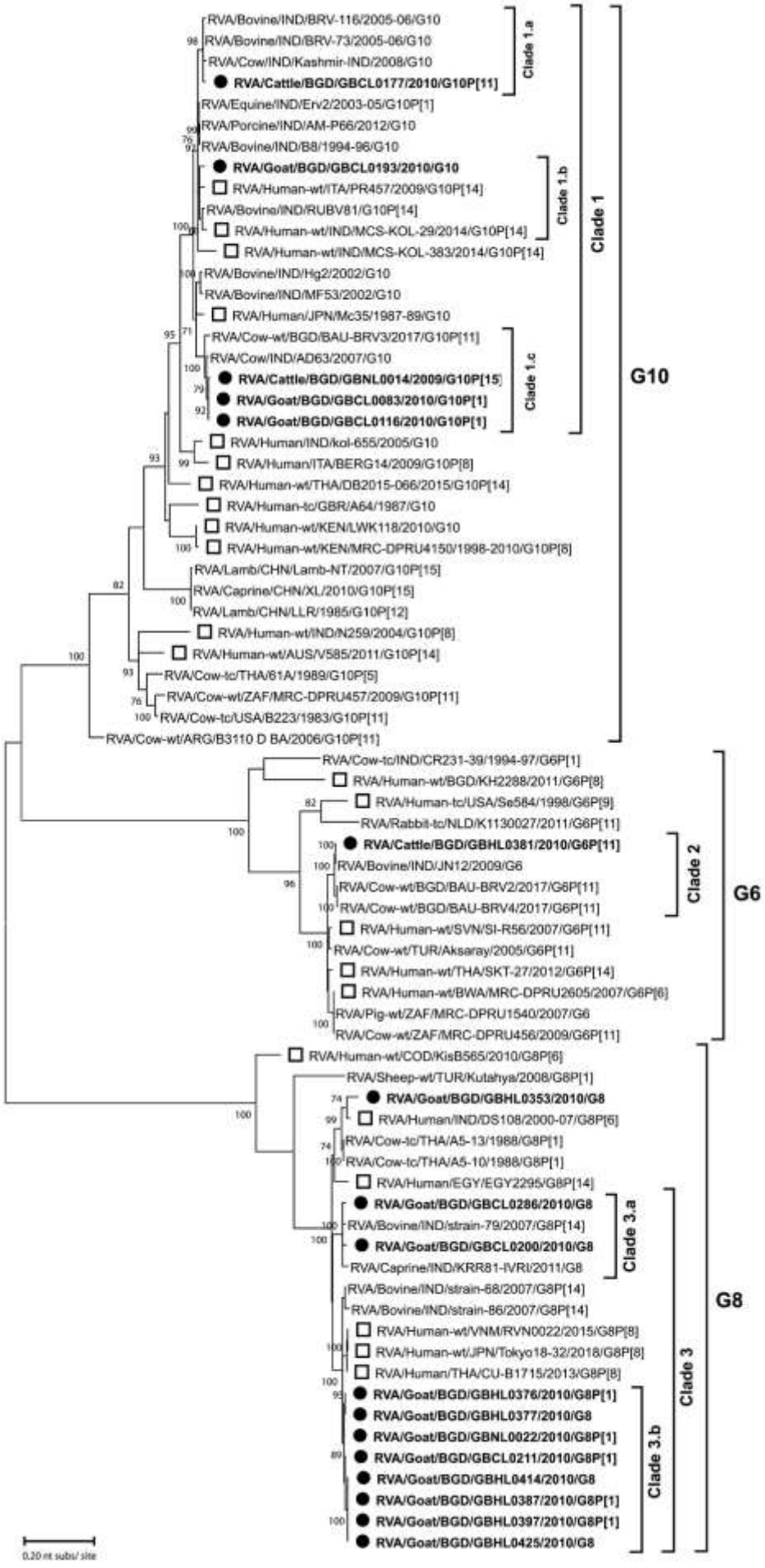
Dinajpur	156	19 (12.2)	1.94 (0.24-15.62)	-
Chattogram	108	26 (24.1)	4.44 (0.56-35.40)	-
Average temperature of months(Cattle)				
Cold months (<=25^{0}C)	69	1 (1.4)	Ref.	-
Warm months (>25^{0}C)	172	14 (8.13)	6.1 (0.78-47.19)	-
Average temperature of months (Goat)				
Cold months (<=25^{0}C)	74	4 (5.4)	Ref.	Ref.
Warm months (>25^{0}C)	205	42(20.5)	4.36 (1.50-12.70)	4.09 (1.41-11.95)*

- *significant at p<0.05

Figure legends

Fig 1. Occurrence of RVA infections (%) among cattle and goats in relation to average temperature of the corresponding months at three veterinary hospitals in Bangladesh during May 2009 and August 2010.

Fig 2. Phylogenetic tree of G-genotype of ruminant rotavirus isolates from Bangladesh. Maximum-likelihood tree constructed using MEGA-X based on nucleotide sequences of the VP7 encoding genes for representative Bangladeshi strains (closed circle) and other strains obtained from GenBank database (human strain indicates with empty square). The evolutionary distances were computed using the T92+G+I method as found best fit. The numbers adjacent to the nodes represent the percentage of bootstrap support when ≥70% (of 1000 replicates) for the clusters to the right of the node.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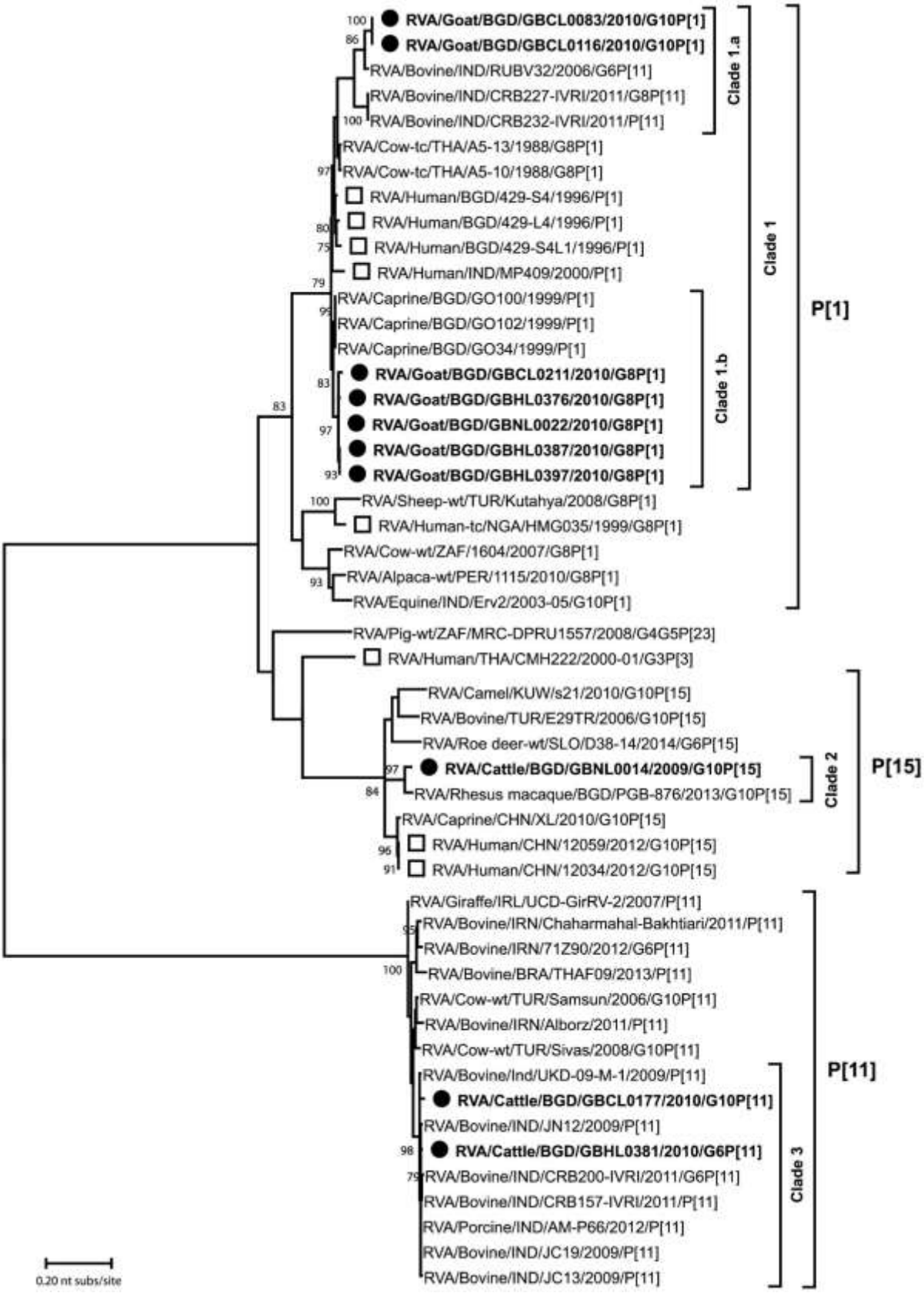
RVA/Cow/IND/Kashmir-IND/2008/G10
RVA/Cattle/BGD/GBCL0177/2010/G10P[11]
Clade 1.a
RVA/Equine/IND/Erv2/2003-05/G10P[1]
RVA/Porcine/IND/AM-P66/2012/G10
RVA/Bovine/IND/B8/1994-96/G10
RVA/Goat/BGD/GBCL0193/2010/G10
RVA/Human-wt/ITA/PR457/2009/G10P[14]
RVA/Bovine/IND/RUBV81/G10P[14]
RVA/Human-wt/IND/MCS-KOL-29/2014/G10P[14]
Clade 1.b
RVA/Human-wt/IND/MCS-KOL-383/2014/G10P[14]
RVA/Bovine/IND/Hg2/2002/G10
RVA/Bovine/IND/MF53/2002/G10
RVA/Human/JPN/Mc35/1987-89/G10
RVA/Cow-wt/BGD/BAU-BRV3/2017/G10P[11]
RVA/Cow/IND/AD63/2007/G10
RVA/Cattle/BGD/GBNL0014/2009/G10P[15]
RVA/Goat/BGD/GBCL0083/2010/G10P[1]
RVA/Goat/BGD/GBCL0116/2010/G10P[1]
Clade 1.c
Clade 1
G10
RVA/Human/IND/kol-655/2005/G10
RVA/Human/ITA/BERG14/2009/G10P[8]
RVA/Human-wt/THA/DB2015-066/2015/G10P[14]
RVA/Human-tc/GBR/A64/1987/G10
RVA/Human-wt/KEN/LWK118/2010/G10
RVA/Human-wt/KEN/MRC-DPRU4150/1998-2010/G10P[6]
RVA/Lamb/CHN/Lamb-NT/2007/G10P[15]
RVA/Caprine/CHN/XL/2010/G10P[15]
RVA/Lamb/CHN/LLR/1985/G10P[12]
RVA/Human-wt/IND/N259/2004/G10P[8]
RVA/Human-wt/AUS/V585/2011/G10P[14]
RVA/Cow-tc/THA/61A/1989/G10P[5]
RVA/Cow-wt/ZAF/MRC-DPRU457/2009/G10P[11]
RVA/Cow-tc/USA/B223/1983/G10P[11]
RVA/Cow-wt/ARG/B3110 D BA/2006/G10P[11]
RVA/Cow-tc/IND/CR231-39/1994-97/G6P[1]
RVA/Human-wt/BGD/KH2288/2011/G6P[8]
RVA/Human-tc/USA/Se584/1998/G6P[9]
RVA/Rabbit-tc/NLD/K1130027/2011/G6P[11]
RVA/Cattle/BGD/GBHL0381/2010/G6P[11]
RVA/Bovine/IND/JN12/2009/G6
RVA/Cow-wt/BGD/BAU-BRV2/2017/G6P[11]
RVA/Cow-wt/BGD/BAU-BRV4/2017/G6P[11]
Clade 2
RVA/Human-wt/SVN/SI-R56/2007/G6P[11]
RVA/Cow-wt/TUR/Aksaray/2005/G6P[11]
RVA/Human-wt/THA/SKT-27/2012/G6P[14]
RVA/Human-wt/BWA/MRC-DPRU2605/2007/G6P[6]
RVA/Pig-wt/ZAF/MRC-DPRU1540/2007/G6
RVA/Cow-wt/ZAF/MRC-DPRU456/2009/G6P[11]
G6
RVA/Human-wt/COD/KisB565/2010/G8P[6]
RVA/Sheep-wt/TUR/Kutahya/2008/G8P[1]
RVA/Goat/BGD/GBHL0353/2010/G8
RVA/Human/IND/DS108/2000-07/G8P[6]
RVA/Cow-tc/THA/A5-13/1988/G8P[1]
RVA/Cow-tc/THA/A5-10/1988/G8P[1]
RVA/Human/EGY/EGY2295/G8P[14]
RVA/Goat/BGD/GBCL0286/2010/G8
RVA/Bovine/IND/strain-79/2007/G8P[14]
RVA/Goat/BGD/GBCL0200/2010/G8
RVA/Caprine/IND/KRR81-IVRI/2011/G8
Clade 3.a
RVA/Bovine/IND/strain-68/2007/G8P[14]
RVA/Bovine/IND/strain-86/2007/G8P[14]
RVA/Human-wt/VNM/RVN0022/2015/G8P[8]
RVA/Human-wt/JPN/Tokyo18-32/2018/G8P[8]
RVA/Human/THA/CU-B1715/2013/G8P[8]
RVA/Goat/BGD/GBHL0376/2010/G8P[1]
RVA/Goat/BGD/GBHL0377/2010/G8
RVA/Goat/BGD/GBNL0022/2010/G8P[1]
RVA/Goat/BGD/GBCL0211/2010/G8P[1]
RVA/Goat/BGD/GBHL0414/2010/G8
RVA/Goat/BGD/GBHL0387/2010/G8P[1]
RVA/Goat/BGD/GBHL0397/2010/G8P[1]
RVA/Goat/BGD/GBHL0425/2010/G8
Clade 3.b
Clade 3
G8
0.20 nt subs/ site

Fig 3. Phylogenetic tree of P-genotype of ruminant rotavirus isolates from Bangladesh. Maximum-likelihood tree constructed using MEGA-X based on nucleotide sequences of the VP4 encoding genes for representative Bangladeshi strains (closed circle) and other strains obtained from GenBank database (human strain indicates with empty square). The evolutionary distances were computed using the T92+G method as found the best fit. The numbers adjacent to the nodes represent the percentage of bootstrap support when ≥70% (of 1000 replicates) for the clusters to the right of the node.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RVA/Goat/BGD/GBCL0116/2010/G10P[1]
RVA/Bovine/IND/RUBV32/2006/G6P[11]
RVA/Bovine/IND/CRB227-IVRI/2011/G8P[11]
RVA/Bovine/IND/CRB232-IVRI/2011/P[11]
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RVA/Cow-tc/THA/A5-10/1988/G8P[1]
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RVA/Human/BGD/429-L4/1996/P[1]
RVA/Human/BGD/429-S4L1/1996/P[1]
RVA/Human/IND/MP409/2000/P[1]
RVA/Caprine/BGD/GO100/1999/P[1]
RVA/Caprine/BGD/GO102/1999/P[1]
RVA/Caprine/BGD/GO34/1999/P[1]
RVA/Goat/BGD/GBCL0211/2010/G8P[1]
RVA/Goat/BGD/GBHL0376/2010/G8P[1]
RVA/Goat/BGD/GBNL0022/2010/G8P[1]
RVA/Goat/BGD/GBHL0387/2010/G8P[1]
RVA/Goat/BGD/GBHL0397/2010/G8P[1]
RVA/Sheep-wt/TUR/Kutahya/2008/G8P[1]
RVA/Human-tc/NGA/HMG035/1999/G8P[1]
RVA/Cow-wt/ZAF/1604/2007/G8P[1]
RVA/Alpaca-wt/PER/1115/2010/G8P[1]
RVA/Equine/IND/Erv2/2003-05/G10P[1]
RVA/Pig-wt/ZAF/MRC-DPRU1557/2008/G4G5P[23]
RVA/Human/THA/CMH222/2000-01/G3P[3]
RVA/Camel/KUW/s21/2010/G10P[15]
RVA/Bovine/TUR/E29TR/2006/G10P[15]
RVA/Roe deer-wt/SLO/D38-14/2014/G6P[15]
RVA/Cattle/BGD/GBNL0014/2009/G10P[15]
RVA/Rhesus macaque/BGD/PGB-876/2013/G10P[15]
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RVA/Bovine/IND/JC19/2009/P[11]
RVA/Bovine/IND/JC13/2009/P[11]
Clade 1.a
Clade 1.b
Clade 1
P[1]
Clade 2
P[15]
Clade 3
P[11]
0.20 nt subs/site