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Short Communication

Molecular Characterization of a Genetically Diverse Bubaline Picobirnavirus Strain, India

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Abstract

Picobirnavirus (PBV) associated gastrointestinal infections have been noticed in humans as well as in a number of animal species, however, to the best of our information, report about the detection and characterisation of PBV in buffaloes is not presented. In this study, RNA polymerase (RdRp) gene targeted RT-PCR assay was used to recognize PBVs and additionally the isolate was analysed for evolutionary and sequence homology. The buffalo PBV isolate was of genogroup I (GG I) nature and a comparative sequence analysis revealed homology of 44.5% and 45.1% at nucleotide and amino acid levels, respectively, with the prototype human PBV strain of GG I (Hu-1-CHN-97/GG-I/2000/AF246939) from China and also with previously reported bovine PBV strain from India (RUBV-P/GG-I/2005/GQ221268). This unique bubaline PBV isolate comprised a separate lineage away from previously reported GG I and GG II PBV sequences. The results provide evidence that this PBV isolate may represent an emerging heterogeneous group of virus with a new lineage.

Keywords: Bubaline, calf, gastroenteritis, Picobirnavirus, RdRP gene, RT-PCR

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บทคัดย่อ

ลักษณะทางโมเลกุลของความหลากหลายทางพันธุกรรมของไวรัส Bubaline Picobirnavirus ในอินเดีย

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เป็นที่น่าสังเกตว่ามีการพบเชื้อ Picobirnavirus (PBV) ที่สัมพันธ์กับการติดเชื้อในระบบทางเดินอาหารในคนใกล้เคียงกับที่พบในสัตว์ แต่อย่างไรก็ตามรายงานและการจำแนกเชื้อ PBV ในกระบือ พบว่ายังมีอยู่ไม่มาก การศึกษานี้ วิธีการทดสอบ RNA polymerase (RdRp) gene targeted RT-PCR ถูกนำมาใช้ในการตรวจหา PBVs และทำการวิเคราะห์ด้านวิวัฒนาการ และลำดับเบสที่คล้ายคลึงกัน พบว่า PBV ที่แยกได้จากกระบือจัดอยู่ในกลุ่ม genogroup I (GG I) จากการวิเคราะห์เปรียบเทียบลำดับพันธุกรรมและลำดับกรดอะมิโน พบความคล้ายคลึงกับสายพันธุ์ต้นแบบที่แยกได้จากคนในประเทศจีน (Hu-1-CHN-97/GG-I/2000/AF246 939) เท่ากับร้อยละ 44.5 และ 45.1 และคล้ายกับสายพันธุ์ PBV ที่แยกได้จากโคในอินเดีย (RUBV-P/GG-I/2005/GQ221268) สายพันธุ์ bubaline PBV ที่แยกได้นี้มีความจำเพาะ ต่างไปจากสายพันธุ์ GG I หรือ GG II ที่เคยรายงานไปก่อนหน้า ผลที่เกิดแสดงให้เห็นว่า PBV ที่แยกได้นี้อาจเป็นเชื้อไวรัสอุบัติใหม่

คำสำคัญ: Bubaline ลูกโค การติดเชื้อในระบบทางเดินอาหาร Picobirnavirus RdRP gene RT-PCR

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Introduction

Viral gastroenteritis is one of the most common diseases distressing young animals globally. Since the first connotation of rotaviruses as the cause of gastroenteritis, information on other viral causes linked with diarrheal diseases in animals has increased gradually, including astroviruses, caliciviruses, coronaviruses and picobirnaviruses. Picobirnavirus (PBV) is an evolving virus and is allied with gastroenteritis in humans and a wide range of animals including pigs, sheep, rabbits, dogs, poultry, monkeys, and snakes (Wang et al., 2007; Carruyo et al., 2008). PBV was also detected as co-infectious agent with rotavirus, calicivirus (Ganesh et al., 2010), astrovirus (Bhattacharya et al., 2006) and *Escherichia coli* (Barreto et al., 2006). As per 9th International Committee on Taxonomy of Viruses (ICTV), PBVs were categorized into a new family *Picobirnaviridae* (Fregolente et al., 2009) and were identified by possessing bi-segmented RNA genome in polyacrylamide gel electrophoresis (PAGE) from a child faecal sample by Pereira et al. (1988). This virus encodes capsid protein (segment 1, 2.2-2.7 kb) and RNA dependent RNA polymerase (RdRp, segment 2, 1.2–1.9 kb). Being highly diverse, PBVs are classified into 2 genogroups, GG I and GG II (Rosen et al., 2000; Bányai et al., 2003, 2008) based on the RdRp gene sequences. Among these two genogroups, GG I PBVs preponderate more than GG II. Indication of concomitant infection having both the groups of PBVs in one host was also testified in humans (Ganesh et al., 2011) and pigs (Smits et al., 2011). Close genetic similarity between animal and human PBVs has also been interpreted by Bányai et al. (2008) and Ganesh et al. (2010), representing zoonotic potential of this isolate.

Compared to human and porcine PBVs, epidemiological studies of bovine PBVs are still in infancy. There are only four reports describing the presence of PBVs in feces of bovines, i.e. two from India (Ghosh et al., 2009; Malik et al., 2011), one from Brazil (Buzinaro et al., 2003) and another from Belgium (Vanopdenbosch and Wellemans, 1989). Evidence is very infrequent regarding occurrence of PBVs in buffalo and its genetic characterization. We describe here the identification, genogrouping and evolutionary analysis of a novel PBV isolate obtained from a diarrheic buffalo calf from India.

Materials and Methods

Clinical material was collected from a buffalo calf suffering from acute enteritis during winter of 2011 in Maharashtra, India. Suspension (10% w/v) of fecal sample in phosphate buffered saline (PBS) (pH 7.4; Sigma-Aldrich, USA) was prepared and filtered through 0.22 µm syringe filter. Viral nucleic acid was isolated from freshly prepared 10% fecal suspension by TriReagent-LS (Sigma-Aldrich, USA).

Manufacturer's protocol was adopted to elute viral RNA in 25 µl of Nuclease Free Water (NFW) and assessed qualitatively and quantitatively using Nanodrop Spectrophotometer (ND-1000, Thermo-Scientific, USA). RNA-PAGE was performed according to methods described in our previous studies (Malik et al., 2011, 2012).

Reverse transcription of RNA was carried out following procedure outlined in our previous studies (Malik et al., 2012). Briefly, cDNA synthesis from freshly extracted viral RNA was performed using Random Hexamer primer (0.2 µg/µl, Fermentas Inc., Glen Burnie, MD). Initially, reaction mixture (100 ng of viral RNA, 0.5 µl Random Hexamer primer, 2 µl Dimethyl sulphoxide) was incubated for 5 min at 95°C followed immediately by snap chilling. Afterwards, addition of 4 µl of 5X RT buffer (Promega, USA), 2 µl of 10 mM dNTPs (Fermentas, MD), 40 U RNase Inhibitor (Ambion, USA), and 200 U Moloney Murine Leukemia Virus (MMLV)-RT (Promega, USA) in the snap chilled PCR tube followed by incubation in a thermocycler at 37^0C for 1 hour (HR-PCR-96G; Haier, Shanghai, China) was carried out. To deactivate residual enzymes, the tube was incubated at 80^0C for 3 min. The cDNA thus obtained was used for PCR and the remaining was kept at -20^0C until further use.

To capture highly diverse PBVs, in-house developed diagnostic RT-PCR targeting conserved region within RdRp gene was performed to get specific amplicon size of 272 bp (data not shown). Further, PCR based genotyping was performed as described by Rosen et al. (2000) and Bányai et al. (2008). The final amplified products (272 bp-diagnostic RT-PCR, 201 bp-genotype I specific RT-PCR) were visualized in ethidium bromide stained 1.5% agarose gel and documented using Transilluminator-UV®300 (UVP Inc, USA). The specific RT-PCR amplicons (272 bp) were purified using QIAquick Gel Extraction kit (Qiagen, Germany) and cloned in to pGEM®-T Easy Cloning vector (Promega, USA). The insert-positive clones were confirmed by sequencing by automated sequencer ABIPRISM®3100 (AME Bioscience, Norway). A dendrogram was constructed by MEGA 5.05 by neighbour-joining statistical method using Maximum Composite Likelihood substitution model with 2000 bootstrap replicates (Tamura et al., 2011). Per cent homology and variance of nucleotide and deduced amino acid sequence were performed to determine percent similarity of PBV genogroup I and II sequences from different geographical locations. The nucleotide sequence of RdRp gene of the buffalo PBV (PBV/GG1/B18/2011) was submitted in the NCBI GenBank database and assigned accession number, JX411967.

Results and Discussion

The initial detection of PBVs relies on migration of its genomic segments in polyacrylamide gel electrophoresis after silver staining (PAGE-ss). Based on electrophoretic mobility of bi-segmented PBV genome in PAGE, two genome profiles have been reported so far viz. large genome profile (Segment 1: 2.3 to 2.6 kbp; Segment 2: 1.5-1.9 kbp) and small genome profile (Segment 1: 1.75 kbp; Segment 2: 1.55 kbp) (Rosen et al., 2000).

In this study, two distinct bands corresponding to larger genome profile of PBV from a buffalo calf faecal sample were visualized in PAGE-ss, specifying the existence of PBV. Comparing the migration pattern with bovine group A rotavirus, the larger band of PBV paralleled segment 2 of rotavirus with size of 2.6 kbp, while smaller band of PBV migrated closer to segment 5 of rotavirus (size 1.6 kbp). Although this preliminary screening by PAGE-ss showed the appearance of similar segment size of the PBV, it did not provide any evidence of genogroups. Reports suggestive of the presence of PBV in a number of hosts and its association with gastroenteritis exist, but data pertaining to its pathogenicity are still limited (Pereira et al., 1989; Ludert and Liprandi, 1993). PBVs have been recovered both from diarrheic and healthy animals. The present findings support an earlier report on PBV detection in diarrheic calves from eastern part of India (Ghosh et al., 2009).

PAGE-ss results confirming the presence of PBV in buffalo calf were further subjected to RT-PCR based genogrouping. The optimized diagnostic RT-PCR conditions yielded a specific amplicon (272 bp) after 35 cycles of amplification. The genogrouping with GG I and GG II primers sets of Rosen et al. (2000) yielded amplification of 201 bp, indicative of GGI nature of the buffalo PBV isolate.

The comparative sequence analysis of ≈245-nt fragment of the RNA-dependent RNA polymerase gene of PBV-B18 isolate revealed less homology (23.5% similarity at nucleotide and 45.1% at amino acid level) with previously reported bovine PBV strain from India (RUBV-P/GG-I/2005/GQ221268). Strikingly, this B18 isolate exhibited less sequence similarity (44.5% and 45.1% homology at nucleotide and amino acid levels, respectively) than expected, with the prototype human PBV genogroup I strain (Hu-1-CHN-97/GG-I/2000/AF246939) from China, while homology with Indian human GG I PBV strains was in the range of 22.2% (AB517735) to 25.4% (AB478502) at nucleotide and 13.7% (AB478502) to 45.1% (AB517735) at amino acid level. Sequence similarity was even lower (19.4%) with segment 2 of the prototype GG II human PBV strain from the USA (Hu-4-GA-91/GG-II/2001/AF246940) and Indian GG II PBVs (22.6%). To determine the evolutionary relationships between buffalo GG I PBV-B18 isolate with cognate genes of different animal and human PBVs, a dendogram was built on the basis of a ≈245-nt fragment of the RNA-dependent RNA polymerase gene (Fig 1). Phylogenetic analysis showed no geographic and host specific clustering of this buffalo PBV and made a separate branch in the phylogenetic tree (Fig 1). The buffalo PBV-B18 isolate made a different phylogenetic clade and did not group with other PBVs according to host species. This finding expands our knowledge on tropism and host range of picobirnaviruses.

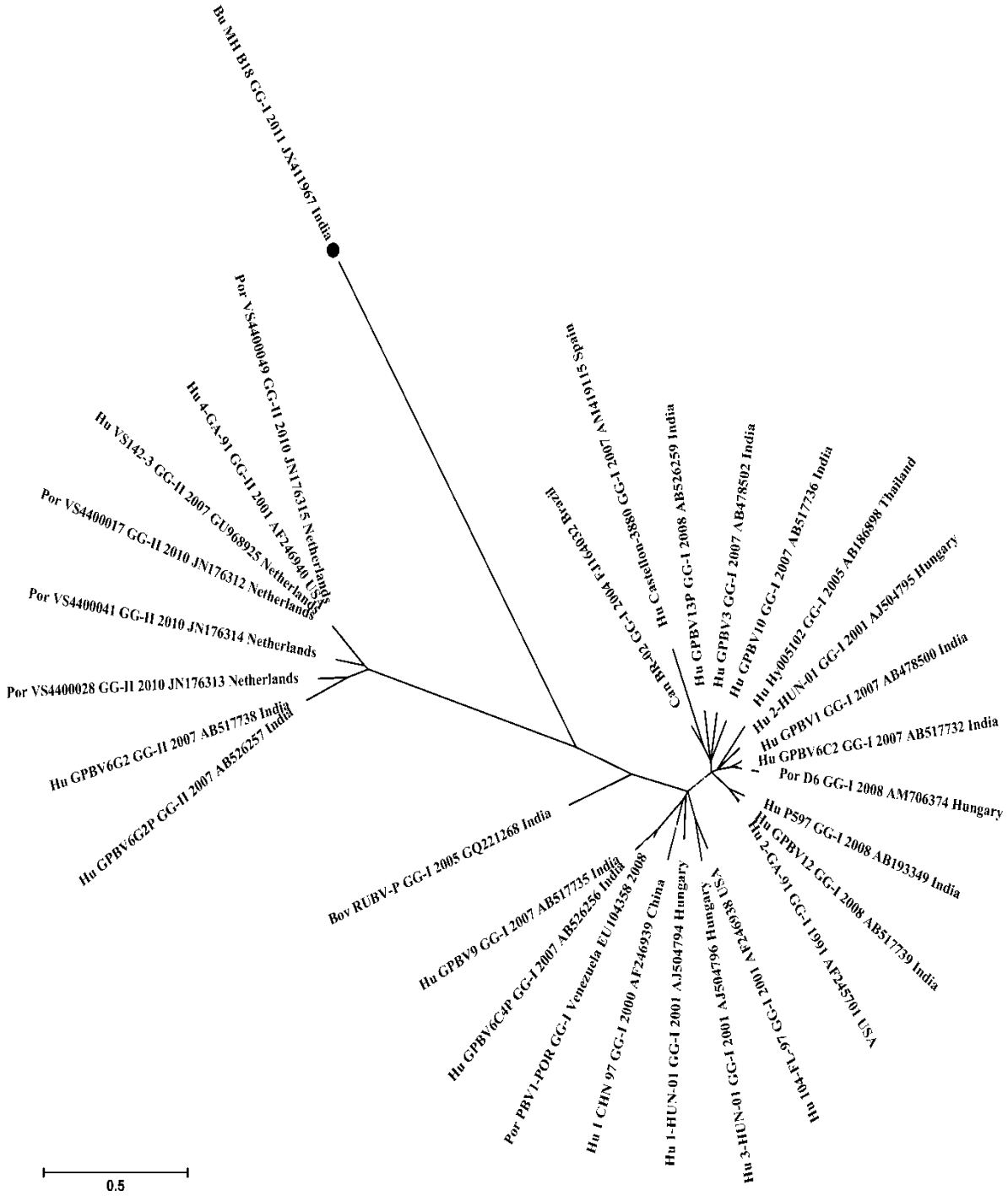

Figure 1 Genetic relatedness based on amino acid sequences of RNA-dependent RNA polymerase (RdRp) of bovine picobirnavirus (PBV) genogroup (GG) 1 (B18) indicated as (●) with other PBV sequences of different host species retrieved from GenBank database (http://www.ncbi.nlm.nih.gov/). Phylogenetic tree was constructed by neighbor-joining (NJ) method implemented in MEGA 5.05 (http://megasoftware.net/). Host species depicted are human (Hu); bovine (Bov); porcine (Por); canine (Can). For each strain, the following data are given: species of origin/strain name/genotypes/year of isolation/accession number/country origin.

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References

Bányai K, Jakab F, Reuter G, Bene J, Uj, M, Melegh B and Szücs G 2003. Sequence heterogeneity among human picobirnaviruses detected in a gastroenteritis outbreak. Arch Virol. 148(12): 2281-2291.

Bányai K, Martella V, Bogdán A, Forgách P, Jakab F, Meleg E, Bíró H, Melegh B and Szucs G 2008. Genogroup I picobirnaviruses in pigs: evidence for genetic diversity and relatedness to human strains. J Gen Virol. 89(2): 534-539.

Barreto ML, Milroy CA, Strina A, Prado MS, Leite JP, Ramos EA, Ribeiro H, Alcântara-Neves NM, Teixeira MG, Rodrigues LC, Ruf H, Guerreiro H and Trabulsi LR 2006. Community-based monitoring of diarrhea in urban Brazilian children: incidence and associated pathogens. Trans R Soc Trop Med Hyg. 100(3): 234-242.

Bhattacharya R, Sahoo GC, Nayak MK, Ghosh S, Dutta P, Bhattacharya MK, Mitra U, Gangopadhyay D, Dutta S, Niyogi SK, Saha DR, Naik TN, Bhattacharya SK and Krishnan T 2006. Molecular epidemiology of human astrovirus infections in Kolkata. India. Infect Genet Evol. 6(6): 425–435.

Buzinaro MG, Freitas PP, Kisiellius JJ, Ueda M and Jerez JA 2003. Identification of a bisegmented double-stranded RNA virus (picobirnavirus) in calf faeces. Vet J. 166(2): 185-187.

Carruyo GM, Mateu G, Martínez LC, Pujol FH, Nates SV, Liprandi F and Ludert JE 2008. Molecular characterization of porcine picobirnaviruses and development of a specific reverse transcription-PCR assay. J Clin Microbiol. 46(7): 2402-2405.

Fregolente MCD, Castro-Dias ED, Martins SS, Spilki FS, Allegretti SM and Gatti MSV 2009. Molecular characterization of Picobirnaviruses from new hosts. Virus Res. 143: 134-136.

Ganesh B, Nagashima S, Ghosh S, Nataraju SM, Rajendran K, Manna B, Ramamurthy T, Niyogi SK, Kanungo S, Sur D, Kobayashi N and Krishnan T 2011. Detection and molecular characterization of multiple strains of picobirnavirus causing mixed infection in a diarrhoeic child: emergence of prototype genogroup II-like strain in Kolkata. India. Int J Mol Epidemiol Genet. 2(1): 61-72.

Ganesh B, Nataraju SM, Rajendran K, Ramamurthy T, Kanungo S, Manna B, Nagashima S, Sur D, Kobayashi N and Krishnan T 2010. Detection of closely related Picobirnaviruses among diarrhoeic children in Kolkata: evidence of zoonoses?. Infect Genet Evol. 10(4): 511-516.

Ghosh S, Kobayashi N, Nagashima S and Naik TN 2009. Molecular characterization of full-length genomic segment 2 of a bovine picobirnavirus (PBV) strain: Evidence for high genetic diversity with genogroup I PBVs. J Gen Virol. 90: 2519-2524.

Ludert JE and Liprandi F 1993. Identification in porcine faeces of a novel virus with a bisegmented double-stranded RNA genome. Arch Virol. 117: 97-107.

Malik YPS, Sharma K, Vaid N, Chakravarti S, Chandrashekar KM, Basera SS, Singh R, Minakshi P, Gulati BR, Bhilegaonkar KN and Pandey AB 2012. Frequency of group A rotavirus with mixed G and P genotypes in bovines: predominance of G3 genotype and its emergence in combination with G8/G10 types. J Vet Sci. 13(3): 271-278.

Malik YS, Chandrashekar KM, Sharma K, Haq AA, Vaid N, Chakravarti S, Batra M, Singh R and Pandey AB 2011. Picobirnavirus detection in bovine and buffalo calves from foothills of Himalaya and Central India. Trop Anim Health Prod. 43(8): 1475-1478.

Pereira HG, de Araujo HP, Fialho AM, de Castro L and Monteiro SP 1989. A virus with bi-segmented double-stranded RNA genome in guinea pig intestines. Mem Inst Oswaldo Cruz. 84: 137–140.

Pereira HG, Flewett TH, Candeias JA and Barth OM 1988. A virus with a bisegmented double-stranded RNA genome in rat (*Oryzomys nigripes*) intestines. J Gen Virol. 69(11): 2749–2754.

Rosen BI, Fang ZY, Glass RI and Monroe SS 2000. Cloning of human picobirnavirus genomic segments and development of an RT-PCR detection assay. Virology. 277(2): 316-329.

Smits SL, Poon LL, van Leeuwen M, Lau PN, Perera HK, Peiris JS, Simon JH and Osterhaus AD 2011. Genogroup I and II Picobirnaviruses in respiratory tracts of pigs. Emerg Infect Dis. 17(12): 2328-2330.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol. 28(10): 2731-2739.

Vanopdenbosch E and Wellemans G 1989. Birna-type virus in diarrhoeic calf faeces. Vet Rec. 125(24): 610.

Wang Y, Tu X, Humphrey C, McClure H, Jiang X, Qin C, Glass RI and Jiang B 2007. Detection of viral agents in fecal specimens of monkeys with diarrhea. J Med Primatol. 36(2): 101-107.