



Occurrence of multiple combinations of G and P types of group A bovine and human rotaviruses in Uttarakhand and Nagaland states, India

Z NONGOTHUNG EZUNG¹, RASHMI SINGH², S P SINGH³, NAVEEN KUMAR⁴ and YASHPAL S MALIK⁵

Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263 145 India
and

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 13 March 2014; Accepted: 6 May 2014

Key words: Bovine, Genotypes, Group A rotavirus, Human, Multiplex-PCR

Rotaviruses (RVs) are important enteric pathogens. India alone accounts for 22% of 453,000 global RV associated deaths among children <5 years annually (Tate *et al.* 2012). The RV associated mortality in animals has not been comprehensively testified in India. So far, 27 G- and 37 P-types of RVA have been reported (<http://rotac.regatools.be/classificationinfo.html>). Although RV infects particular species, heterologous infections may occur. Rotaviruses are continuously evolving due to lack of proofreading activity in RNA polymerase and segmented nature of genome which leads to frequent reassortment (Malik *et al.* 2013). Sequencing of whole genome of RVs has shed light into complex events of interspecies transmission and reassortment among human and bovine RVs. Owing to high genetic diversity among RVs, present study was undertaken to know circulation status of different genotypic combinations of RVA among bovine and human population in two states of India (Uttarakhand and Nagaland) during 2010–2012.

Diarrhoeic faecal samples (150) from cattle calves (0–3 months) were collected from dairy farms in Pantnagar (Uttarakhand) during December-January in 2010–11 and 2011–12. Diarrhoeic stool samples from human infants (0–5 years), were obtained from Dr Sushila Tiwari Memorial Hospital, Haldwani, Uttarakhand (n=28) during December-January 2011–2012 and Civil Hospital Kohima, Nagaland (23) during June-July 2011. A 10% faecal suspension (w/v) was prepared in phosphate buffer saline.

Total RNA was extracted following protocols optimized earlier (Basera *et al.* 2010). RNA-PAGE was done as per standard procedure (Laemmli 1970). A commercially available ELISA kit was used to screen bovine samples.

Present address: ¹SMS (nongoniel@gmail.com), Animal Science, KVK, Nagaland University, Lumami, Zunheboto, Nagaland. ²Associate Professor (madan_rs@rediffmail.com), DUVASU, Mathura. ³Professor (dr.sureshvph@gmail.com), GBPUAT, Pantnagar. ⁴PhD Scholar (navyog.yadav84@gmail.com), ⁵Senior Scientist (malikyps@yahoo.com), Division of Biological Standardization, IVRI.

cDNA synthesis and amplification of full length VP7 gene (1062 bp) and partial length VP4 gene (864 bp) was performed as described earlier (Malik *et al.* 2012). Bovine RVA genotyping was performed using G3, G6, G8, G10, P[1], P[5] and P[11] typing primers (Iturriza-Gomara *et al.* 2004). Human RVA genotyping was performed using G1, G2, G3, G4, P4, P8, and P9 genotype specific primers (Iturriza-Gomara *et al.* 2004).

A typical 4: 2: 3: 2 migration pattern characteristic of RVA was observed in 17.33% bovine and 15.68% human samples. Prevalence of bovine RVA (26/150) is higher than reported in this region. Basera *et al.* (2010) reported 10.15% bovine RVA and Malik *et al.* (2014a) detected only 8 samples positive for bovine RVA out of 87 in Hilly areas of Uttarakhand. This may be attributed to period of sampling as peak winter months were chosen when incidence is maximum. All bovine RVA were long electropherotypes having two patterns. In type A segments 1, 2, 3 and 4 migrated separately and in Type B segment 2 and 3 co-migrated (Fig. 1a). 7 and 8 moved as a single segment in all. Basera *et al.* (2010) reported all 13 bovine RVA as long electropherotypes having 1–4 migrating separately, 2 and 3 co-migrating and 7–9 as single segment. Malik *et al.* (2012) detected all 17 bovine isolates as long electropherotypes with 7, 8 and 9 segments co-migrating.

In human RVA, 14.28% (4/28) were from Haldwani, Uttarakhand and 17.39% (4/23) were from Kohima, Nagaland. Both long and short electropherotypes were detected (Fig. 1b). No distinct seasonal variation was observed as samples were taken in winter and dry months in Haldwani and summer and humid months in Kohima. The prevalence was less as compared to a study in another North Eastern State, Manipur where Mukherjee *et al.* (2010) detected ~ 50% human RVA. They found no distinct seasonal variation but a high frequency in children of 3–23 months age group. Dash *et al.* (2012) reported 46/220 samples positive for human RVA in diarrhoeic children from Uttarakhand and Uttar Pradesh and detected both long and short electropherotypes.

In ELISA, 22% (33/150) bovine samples were confirmed

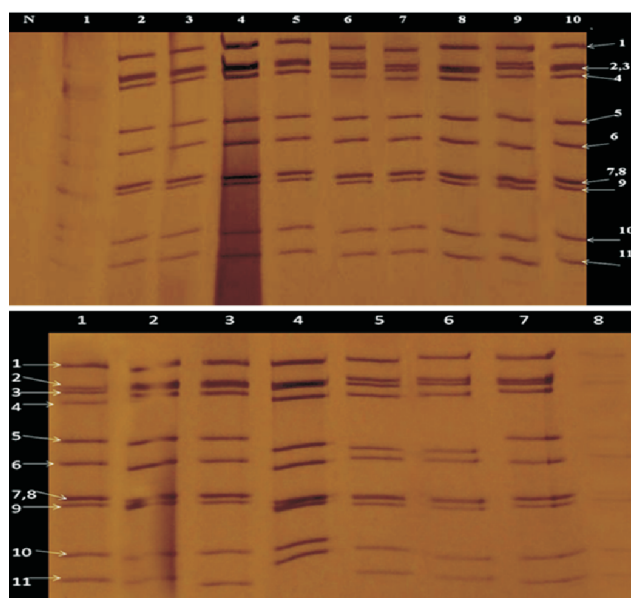


Fig. 1a–b. **a.** RNA PAGE showing electrophoresis and migration pattern of Bovine rotavirus; Lane N: Negative Control; Lane 1–10: BRV positive samples; Lane 5, 6, 7, 8 and 9: Type A migration pattern (segments 1,2,3 and 4 migrated separately) Lane 1, 2, 3, 4 and 10: Type B migration pattern (segments 2 and 3 comigrated). **b.** RNA PAGE showing electrophoresis and migration pattern of Human rotavirus; Lane 1–8: HRV positive samples; Lane 1, 5, 6 and 7: Type A (segments 1, 2, 3 and 4 migrated separately); Lane 2, 3, 4 and 8: Type B (segments 2 and 3 comigrated)

positive while 31/150 (20.66%) bovine samples and 8/51 (15.68%) human samples were positive by VP6 gene specific RT-PCR. This indicates ELISA and VP6 gene specific RT-PCR being the more sensitive assays than RNA-PAGE.

The first round RT-PCR from bovines yielded 1062 bp and 864 bp amplicons for VP7 and VP4 genes, respectively. The second round PCR produced amplicons of 373, 581 and 397 bps for G3, G6 and G10 genotypes, respectively (Fig. 2). Mixed G3G10 and G3G6G10 types were also observed. P typing produced amplicons 334 bp and 453 bp corresponding to P[11] and P[1], respectively (Fig. 3). P[5] was not detected in any isolate. Among typed bovine samples, G3 and G10 were most predominant G types (23.5%). Mixed G types (G3G10, G3G6G10) were identified as 52.93%. Gulati *et al.* (1999) detected G10 (83%) as most predominant bovine G type in India. In a recent study on BRV from North India, Manuja *et al.* (2008) showed 73.3% as G10 type and 26.7% as G6 genotype. In earlier reports, Basera *et al.* (2010) detected G3 as the most predominant G-type in different regions of Uttarakhand. Malik *et al.* (2012) showed that 52.9% (9/17) bovine RVAs were G3 and 47% were mixed G types as 35.3% G3G8 (6/17) and 11.8% G3G10 (2/17) in the temperate region of Western Himalaya. In present study, P[1] and P[11] were most predominant P types (23.07%) and 38.46% exhibited dual type (P[1]P[11]). This study is in conformity with earlier report by Malik *et al.* (2012), where P types were

mostly P[11] (94.1%) while 5.9% were the dual type (P[1]P[11]) in temperate region of Western Himalaya.

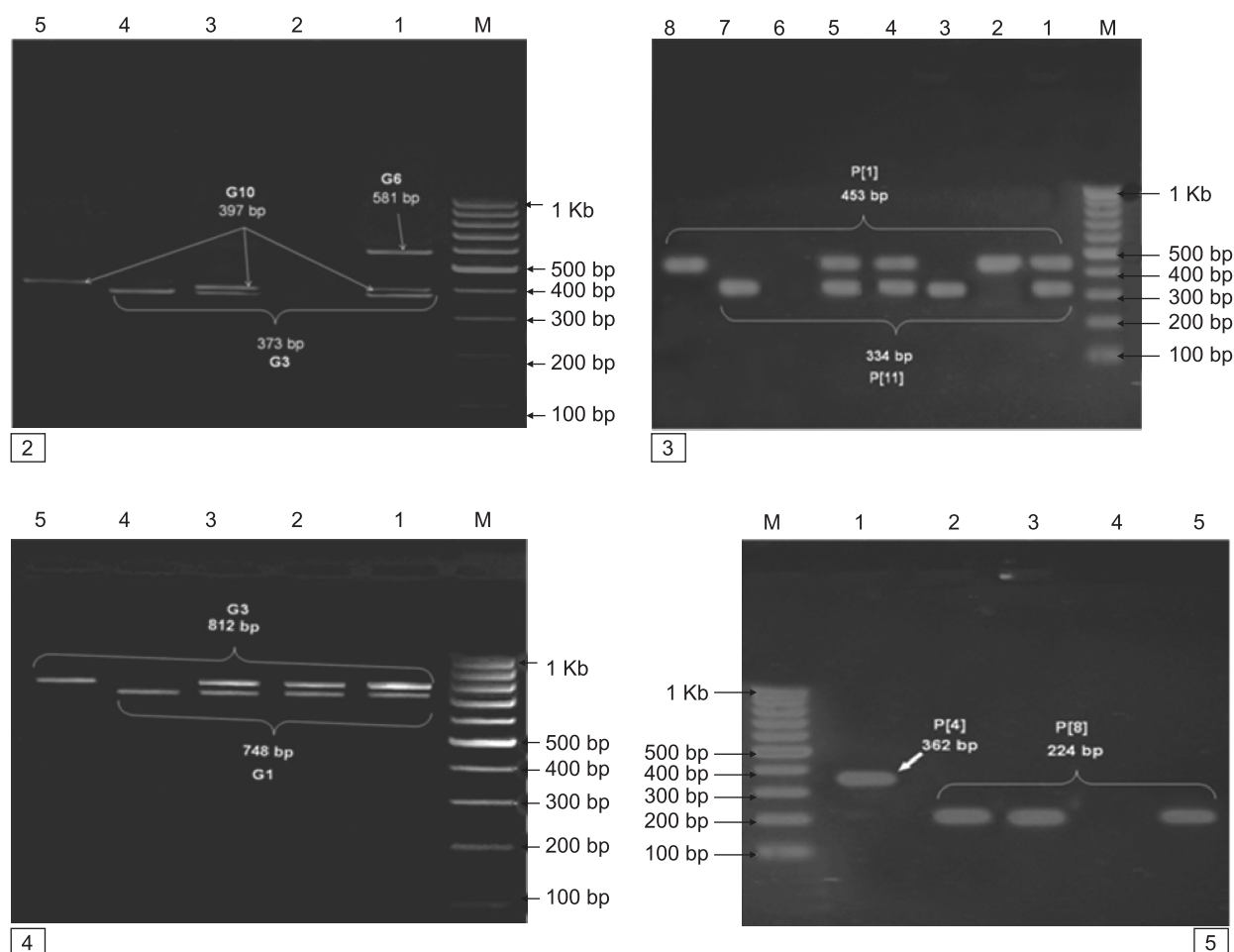
In human samples, 1062-bp amplicons for VP7 and 877-bp product for VP4 were obtained. G typing produced amplicons of 748 and 812 bps for G1 and G3, respectively (Fig. 4). None of samples was positive for G2 and G4. Mixed G1G3 genotype was also observed. P typing produced amplicons 362 and 224 bps, corresponding to P[4] and P[8], respectively (Fig. 5). P[9] was not detected in any isolate. Among typed human samples, 28.57% were G1, 14.28% were G3 and 57.14% were mixed (G1G3) genotypes. P[4] was 14.28% and P[8] was 42.85%. In Uttarakhand, G1 and G3 were 14.28%, G1G3 was 57.14%, P[4] was 14.28% and P[8] was 28.56%. In Nagaland, G1 and P[8] were 14.28%. Presence of G1 as most predominant type is in conformity with Mukherjee *et al.* (2010) who reported G1 as most predominant type (51.63%), followed by G2 (23.36%) and G12 (8.19%) in Manipur. Their P genotype analysis showed P[8] as predominant genotype (39%) followed by P[4] (30%) and P[6] (17.6%). No such data is available on human RVA types in Uttarakhand for comparison. Though this appears to be first report on detection of human RVA from the region, molecular evidences of mixed viral infection with human RVA and Picobirnavirus in an infant hospitalized with acute gastroenteritis in Uttarakhand have been reported (Malik *et al.* 2014b).

The most predominant G and P type combination in bovine samples was 23.07% G3G6G10P[11] followed by 15.38% each of G3P[11], G10P[1]P[11], G3G10P[1]P[11] and G3G6G10P[1], while 7.69% had G10P[1] and G3P[1]P[11] combination (Table 1). This is in contrast with earlier report by Gulati *et al.* (1999) where G10P[11] (81%) was most predominant combination among Indian bovine population, followed by G6P[1] (3%) and G6P[11] (3%). Malik *et al.* (2012) detected G3P[11] as most prevalent genotype and mixed genotypes as G3G10P[11], G3G8P[11], G3G8P[1]P[11] and G3G10P[1]P[11].

In HRV, most predominant G and P type combination was G1G3P[8] (28.57%) followed by G1P[4] (14.28%) from Uttarakhand and G3P[8] (14.28%) from Nagaland. Mukherjee *et al.* (2010) reported G1P[8] as most common strain (36%) followed by G2P[4] (22%), G12P[6] (8%) and G9P[6] (3%) in human RVA circulating in Manipur.

In present study, only 17 bovine and 7 human samples were typed and rest remained non typeable. Similar were findings of bovine RV G typing where Basera *et al.* (2010) failed to amplify viral gene despite being known to contain sufficient rotaviral particles by RNA-PAGE and sandwich ELISA. In a study conducted by Hussain *et al.* (1996), neither P9 nor P10 genotype was detected using type specific primers.

Overall, results confirm a changing pattern of circulation of group A RVs in India. The detection of bovine G3, G10, P[1], P[11] types and human G1, G3, P[4], P[8] provides significant information on increasing trend of these genotypes. G3 was reported as most predominant bovine



Figs 2–5. **2.** Multiplex PCR for genotyping of VP 7 gene in Bovine rotavirus; Lane M: GeneRuler™ 100 bp plus DNA Ladder (MBI, Fementas); Lane 1–5: BRV isolates. **3.** Multiplex PCR for genotyping of VP 4 gene in Bovine rotavirus; Lane M: GeneRuler™ 100 bp plus DNA Ladder (MBI, Fementas); Lane 1–8: BRV isolates. **4.** Multiplex PCR for genotyping of VP 7 gene in Human rotavirus; Lane M: GeneRuler™ 100 bp plus DNA Ladder (MBI, Fementas); Lane 1–5: BRV isolates. **5.** Multiplex PCR for genotyping of VP 4 gene in Human rotavirus; Lane M: GeneRuler™ 100 bp plus DNA Ladder (MBI, Fementas); Lane 1–5: BRV isolates

type in Uttarakhand but based on this study G10 has emerged as a major type circulating in this region. The study on human G and P types have reinforced previous described types and also provided insight into types circulating in two hill states Uttarakhand and Nagaland where reports are scanty. There are 45 different combinations of G and P types that have been recognized (Mukherjee *et al.* 2011a). The present study, has given newer combinations and emphasized the presence of more number of G types circulating together. In human isolates from Manipur, India, rotavirus strains were found to possess porcine or bovine characteristics (Mukherjee *et al.* 2011b) indicating possible zoonotic transmission. Therefore, further work is needed to completely characterize the isolates in the present study to get the phylogenetic data.

SUMMARY

The present study describes the distribution of RV in bovine and human population from Uttarakhand and North eastern region of India. Group A rotavirus (RVA) was detected in 17.33% and 15.68% diarrhoeic cases from

bovine and humans, respectively. All bovine RVA isolates were long electropherotypes whereas, in human RVA isolates both long and short electropherotypes were detected. Genotyping by multiplex RT-PCR revealed presence of G3 and G10 genotypes in bovine and G1 and G3 in humans. Among P types, P[1] and P[11] in bovine and P[4] and P[8] in human were detected. The G and P type combinations present were G3P[11], G10P[1], G3P[1]P[11], G10P[1]P[11], G3G10P[1]P[11], G3G6G10P[1] and G3G6G10P[11] in bovine, while G1P[4], G3P[8] and G1G3P[8] in human samples.

REFERENCES

- Basera S S, Singh R, Vaid N, Sharma K, Chakravarti S and Malik Y P S. 2010. Detection of rotavirus infection in bovine calves by RNA-PAGE and RT-PCR. *Indian Journal of Virology* **21** (2): 144–47.
- Dash S K, Kumar K, Tewari A, Varshney P, Goel A and Bhatia A K. 2012. Detection of rotavirus from hospitalized diarrheic children in Uttar Pradesh, India. *Indian Journal of Microbiology* **52** (3): 472–77.

- Gulati B R, Nakagomi O, Koshimura Y, Nakagomi T and Pandey R. 1999. Relative Frequencies of G and P Types among rotaviruses from Indian Diarrheic Cow and Buffalo calves. *Journal of Clinical Microbiology* **6**: 2074–76.
- Hussain M. 1996. 'Study of variation in rotavirus gene segments 4 and 9 by Polymerase Chain Reaction (PCR) and restriction enzyme analysis'. Ph.D. Thesis submitted to AIIMS, Delhi, India.
- Iturriza-Gómara M, Kang G and Gray J. 2004. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. *Journal of Clinical Virology* **31**(4): 259–65.
- Laemmli U K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227** (5259): 680–85.
- Malik Y P S, Sharma K, Vaid N, Chakravarti S, Chandrashekar K M, Basera S S, Singh R, Minakshi, Prasad S, Gulati B R, Bhilegaonkar K N and Pandey A B. 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. *Journal of Veterinary Science* **13** (3): 271–78.
- Malik Y P S, Sharma K, Vaid N, Batra M, Prasad M, Prasad G, Gulati B R and Bhilegaonkar K N. 2013. Designing and validation of new primers for efficient genotyping of animal G3 rotaviruses. *Veterinarski Arhiv* **83** (6): 611–22.
- Malik Y P S, Sharma K, Kumar N, Haq A A and Vaid N. 2014a. Determination of bovine rotavirus genotypes (G and P) circulating in hilly region of Uttarakhand (2010–2012) by polymerase chain reaction. *Indian Journal of Animal Sciences* **84** (2): 152–55.
- Malik Y P S, Rawat V, Sharma K, Vaid N, Kumar N, Sharma A K, Rakholia R and Ganesh B. 2014b. Molecular evidences of mixed viral infection in an infant hospitalized with acute gastroenteritis in northern India. *Journal of Pure and Applied Microbiology* **8**(1): 733–37.
- Manuja B K, Prasad M, Manuja A, Gulati B R and Prasad G. 2008. A novel genomic constellation (G10P[3]) of group A rotavirus detected from buffalo calves in northern India. *Virus Research* **138** (1–2): 36–42.
- Mukherjee A, Chattopadhyay S, Bagchi P, Dutta D, Singh N B, Arora R, Parashar U D, Gentsch J R, Chawla-Sarkar M. 2010. Surveillance and molecular characterization of rotavirus strains circulating in Manipur, North-Eastern India: increasing prevalence of emerging G12 strains. *Infection, Genetics and Evolution* **10**: 311–20.
- Mukherjee A and Chawla-Sarkar M. 2011a. Rotavirus Infection: A Perspective on Epidemiology, Genomic Diversity and Vaccine Strategies. *Indian Journal of Virology* **21**(1): 11–23.
- Mukherjee A, Ghosh S, Bagchi P, Dutta D, Chattopadhyay S, Kobayashi N and Chawla-Sarkar M. 2011b. Full genomic analyses of human G4P[4], G4P[6], G9P[19] and G10P[6] strains from North-eastern India: evidence for interspecies transmission and complex reassortment events. *Clinical Microbiology and Infection* **17** (9): 1343–46.
- Tate J E, Burton A H, Boschi-Pinto C, Steele A D, Duque J and Parashar U D. 2012. 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 Disease* **12** (2): 136–41.