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Detection and characterization of caprine and ovine rotaviruses, India

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Rotaviruses (RVs), member of the family *Reoviridae*, are important enteric pathogens. The small ruminants play an important role in microeconomics of rural communities in developing countries. Infectious diseases of caprine and ovine inflict a heavy loss at national level (Kumar *et al.* 2013). Studies on prevalence of RV infections in these animal species, with determination of circulating genotypes, are still limited globally. In India, existing reports on ovine RVs have been largely confined to hilly regions (Wani *et al.* 2004a, Wani *et al.* 2004b, Wani *et al.* 2007, Gazal *et al.* 2011). Prevalence of caprine RV and its association with outbreaks of diarrhea have been reported (Munoz *et al.* 1995, Munoz *et al.* 1996, Pratelli *et al.* 1999, Talukder 1999, Dey *et al.* 2007, Khafagi *et al.* 2010, Alkan *et al.* 2012, Papp *et al.* 2014) with a single report from India (Reddy *et al.* 2014).

Molecular characterization studies have revealed genetic relatedness of animal and human RV strains, even indicating anthroponotic transmission (Ghosh *et al.* 2010, Rajendran and Kang 2014, Doro *et al.* 2015). In India, intensified surveillance of RV strains has been carried out in humans and bovines, but there is complete paucity of literature on caprine and ovine RV of Indo-Gangetic Plain and region adjunct. The present study determined the prevalence of RV in small ruminants in this region.

Diarrheic fecal samples (225) were collected from lambs (100) and kids (125) of 0–3 months old during July 2013 - Feb 2014 from unorganized (small holding farmers) and organized sheep and goat farms in the South Western Upper Gangetic Plain, India. Ten percent fecal suspension (w/v) prepared in phosphate buffered saline (PBS, pH 7.2) was used for primary screening of RV genome by RNA – PAGE as per protocol described by Malik *et al.* (2012). Rotavirus antigen detection ELISA kit (Bio K-343/2, Bio-X Diagnostics, Belgique) was used to screen the fecal samples as per the manufacturer's instructions. Sample that gave a percentage of 7.34% or above was considered as positive. Reverse transcription-PCR for amplifying VP6 gene and multiplex PCR for genotyping of group A rotavirus was done using primers custom synthesized from Integrated DNA Technologies Inc., Iowa, USA as given in Table 1 as per Basera *et al.* (2010) and Malik *et al.* (2012).

All diarrheic fecal samples screened were negative for RV genome in RNA-PAGE. Few samples exhibited truncated banding pattern. In RVA specific ELISA, only one sample from a diarrheic kid (K23) was positive, showing a net OD (at 450 nm) as 0.232 and a percentage value of 8.05, while all ovine samples were negative. In VP6 gene specific RT-PCR, an amplicon of expected 227 bp product was obtained in 10 caprine and 5 ovine samples (Fig. 1) thus, giving a prevalence of 8% and 5% of caprine and ovine RVA, respectively. Caprine RVA was in 1–4 weeks old kids and ovine RVA dominated in 2–3 weeks old lambs, both in samples from small holdings. On genotyping with G-specific primers, amplicon size of 746 bp and 590 bp corresponding to G6 and G8 genotypes was obtained in one caprine sample (K23) (Fig. 2) while remaining 14

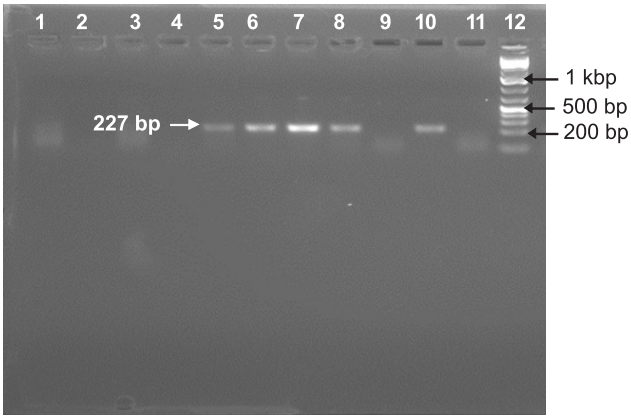

Fig. 1. Screening of ovine and caprine fecal samples by RT-PCR for VP6 gene of group A rotavirus. Lane 12, 1 kb plus DNA Ladder (MBI, Fermentas). Lanes 5-8, 10, Positive RVA samples showing 227 bp amplified VP6 gene. Lanes 1-4, 9, 11: Negative RVA samples.

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Table 1. Primers used for detection of group A rotavirus

Gene	Primers	Sequence (5' to 3')	Position
VP6	GARV-D-VP6-F	TTTGATCACTAAYTATTCACC	1130–1150
	GARV-F-VP6-R	GGTCACATCCTCTCACTA	1339–1356
VP7	Beg9-F	GGCTTTAAAAGAGAGAATTTCCGTCTGG	1–28
	End9-R	GGTCACATCATACAATTCTAATCTAAG	1062–1036
VP4	Bov4Com 5-F	TTCATTATTGGGACGATTCACA	1064–1085
	Bov4Com 3–R	CAACCGCAGCTGATATATCATC	1897–1918
Genotype			
	End9-R	GGTCACATCATACAATTCTAATCTAAG	1062–1036
G3(GOM)	GARV-G3	CTAATTCANCACARGAAG	250 to 267
G5 (S)	GARV-G5	TAGGRTGTTCGACTACAGAC	653 to 672
G6 (S)	GARV-G6	CAAACGAAATAGCTGATACCGAA	311 to 333
G8 (S)	GARV-G8	ATGAAGTATAATGCYAATTCAGA	471 to 493
G10 (1)	RVG10	ATGTCAGACTACARATACTGG	666–687
	Bov4Com 5-F	TTCATTATTGGGACGATTCACA	1064–1085
P[1]	GARV-VP4 P[1]	TTAAATTCATCTCTTAGTTCTC	1505–1526
P[5]	GARV-VP4 P[5]	GGCCGCATCGGATAAAGAGTCC	1704–1725
P[11]	GARV-VP4 P[11]	TGCCTCATAATATTGTTGGTCT	1377–1398

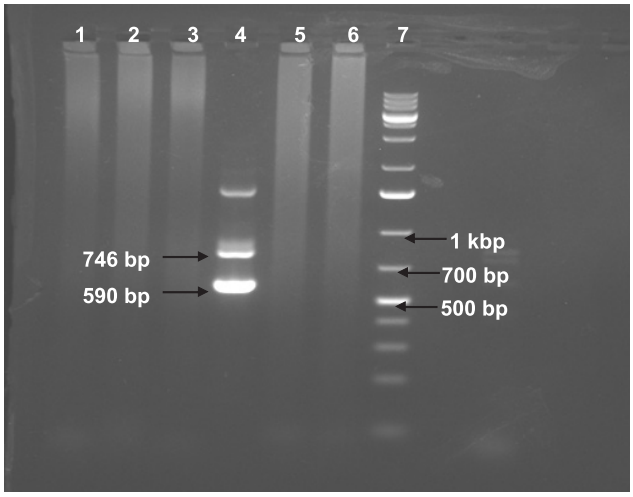

Fig. 2. G typing of group A rotavirus by multiplex-nested PCR. Lane 7, 1 kb plus DNA Ladder (MBI, Fermentas). Lane 4, K23 positive for G6 (746 bp) and G8 (590 bp).

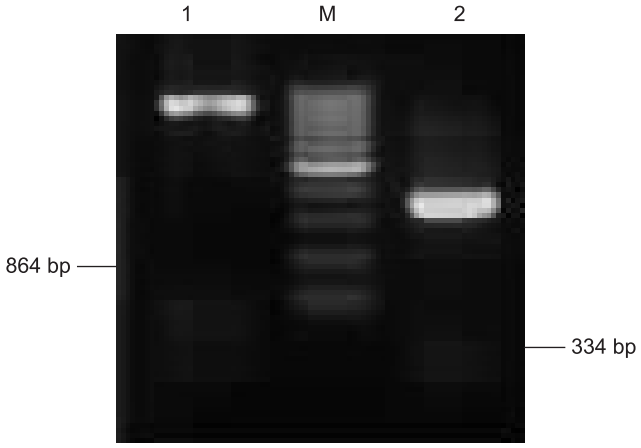

Fig. 3. P typing of group A rotavirus by multiplex-nested PCR. Lane M, 1 kb plus DNA Ladder (MBI, Fermentas). Lane 1, K23 showing 864 bp amplicon for partial length VP4 gene. Lane 2, K23 showing amplified product of 334 bp for P [11].

samples remained untypeable. Similarly, on P-typing, amplicon size of 334 bp corresponding to P [11] was obtained in the same caprine isolate (K23) (Fig. 3) and rest of the samples remained untypeable. In the present study, the single caprine sample (K23) typed was having mixed genotype as G6G8P[11] combination.

The absence of clear 11 segments of RV genome with truncated pattern may indicate less number of rotavirus particles in feces to be detected clearly in PAGE. In similar reports, 21.7% caprine RVA samples detected in ELISA failed to give characteristic RVA electropherotype (Ali *et al.* 2011). In another study, RVA was detected in 25% (24/129) diarrheic lambs, although, 21 were positive in ELISA and only 7 in PAGE (Wani *et al.* 2004a). The sensitivity of ELISA over PAGE has been reported as 10^6 and 10^{11} rotavirus particles per ml, respectively (Arguelles *et al.* 2000). Similar to present findings, caprine RV was detected in 8.68% cases between 7 days–1 month old diarrheic kids of Black Bengal goats in Mymensingh, Bangladesh (Dey *et al.* 2007). Group A rotaviruses was detected in 8.1% diarrheic goat kids in Spain with highest prevalence in one-week to one-month age group (Munoz *et al.* 1996); 6.18% rotavirus infection was reported in goat kids in Bangladesh (Talukder 1999); 7.9% RVA was reported in kids from Egypt (Khafagi *et al.* 2010). In contrast to present finding, a prevalence of 15.0 and 13.33% for RVA was reported by RT-PCR and RNA-PAGE, respectively, in diarrheic kids in organized farms between 0–1 month age group (Reddy *et al.* 2014). Gazal *et al.* (2011) reported 13.46% prevalence of ovine RVA showing a characteristic 4–2–3–2 migration pattern in PAGE. In the present study, low prevalence of ovine RV was observed, indicating that ovines are less frequently infected than caprines. Similar finding was reported by Munoz *et al.* (1996) where prevalence of RVA was higher in goats than sheep. Whereas, Khafagi *et al.* (2010) reported 12.3% and 7.9% prevalence rate of RV

infection in lamb and kids, respectively. None of the ovine sample could be typed in the present study although, G6P[11] has been observed in Jammu & Kashmir in earlier reports (Gazal *et al.* 2012). The finding of mixed genotype G6G8P[11] in the present study was not reported earlier. These mixed infections most likely represent naturally occurring reassortmant of rotavirus strains. G6 and G8 types have been reported as single genotype (Khafagi *et al.* 2010). Pratelli *et al.* (1999) identified G6P6[1] caprine RVA in two strains isolated in Italy. Alkan *et al.* (2012) characterized the caprine strain (RVA/goat-tc/TUR/Kirklareli/2007/G8P[1]) as G8P[1] described previously in lambs (Pratelli *et al.* 1999, Galindo-Cardiel *et al.* 2011). Lee *et al.* (2003) identified the first G3P5[3] caprine RVA in Korean diarrheic goat kid. G6P[1] was identified in three goat RVA strains in Bangladesh and the full genome of one of these strains was sequenced (Ghosh *et al.* 2010). Uriarte *et al.* (2014) detected first caprine rotavirus as G8P[1] in Argentina displaying genomic features resembling virus strains infecting members of the *Bovidae* and *Camelidae*. In the present study, a large number of samples remained untypeable. The reason could be use of typing primers from bovine studies because of scanty literature on caprine and ovine strains and diversity among ovine and caprine RV from bovine RVs. Also, genotype specific PCR primers have failed to amplify VP7 and VP4 genes of global common strains (Adah *et al*.1997, Iturriza-Gomara *et al.* 2000, Banyai *et al.* 2005).

RVs from each animal species have predominant and intrinsic G-type(s) and P-type(s) (Kobayashi *et al.* 2007). The predominant G-types and P-types in cattle are common to those in sheep/goat, suggesting the high frequency of RV transmission between these two animal species. The present G6 and G8 types are common in bovines and thus require complete genomic constellation to study genetic relatedness and their zoonotic relevance.

SUMMARY

In this study, we report detection, prevalence and genotyping of caprine and ovine rotaviruses. Diarrheic fecal samples (225) of 0–3 months old lambs and kids were screened using RNA-PAGE, ELISA and VP6 gene specific RT-PCR assay. All the fecal samples failed to reveal a clear 11 segmented banding pattern of RV genome in PAGE. In sandwich-ELISA for group A rotavirus (RVA), only one caprine sample was detected positive. In RT-PCR, 10 (8%) caprine samples and 5 (5%) ovine samples were positive for RVA. In multiplex nested PCR based genotyping for VP7 (G) and VP4 (P) genes, one of the sample from caprine possessed G6G8P[11] genotype combination. This is possibly the first report on caprine rotavirus type in India, although a limited number have been characterized outside India. None of the ovine sample could be typed in this study, though one report of genotyping is available in India. The study aimed to intensify surveillance in earlier ignored species for RV because of strong genetic relatedness between animal and human strains and possible transmission.

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