Complete genome characterization of a rotavirus B (RVB) strain identified in Alpine goat kids with enteritis reveals inter-species transmission with RVB bovine strains

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Abstract
Rotavirus B (RVB) has been associated with enteric disease in many animal species. An RVB strain was identified in pooled intestinal samples from Alpine caprine kids (between 2 and 3 days of age) experiencing high (>90 %) morbidity, and the complete caprine RVB genome was characterized. Histology revealed villus atrophy, the samples tested positive for RVB by real-time RT-PCR and metagenomic next-generation sequencing identified only RVB and orf virus. In the VP4 gene segment, the caprine RVB strain had a higher percentage nucleotide identity to the Indian bovine RVB strains than to the Japanese bovine RVB strains, but the VP7, VP6, VP2, NSP1, NSP2 and NSP5 gene segments of the American caprine RVB strain were genetically related to the Japanese bovine RVB strains. The results indicate a lack of RVB sequences to understand reassortment or the evolutionary relationship of RVB strains from cattle and goats.
Table 1. Comparison of segment size and nucleotide and amino acid sequences identities (percentages) of the caprine RVB strain with those of the bovine, porcine, human and rat RVB strains

| Gene segment | Bovine nt | Bovine aa | Porcine nt | Porcine aa | Human nt | Human aa | Rat nt | Rat aa |
|--------------|-----------|-----------|------------|------------|----------|----------|--------|-------|
| VP7 (744, 247) | 77.3      | 99.7      | 98.1       |             | 98.1     |          | 58.3   | 69.9   |
| VP4 (2277, 758) | 89.1      | 99.7      | 93.0       | 93.4       | 93.0     |          | 68.4   | 61.9   |
| VP6 (1176, 391) | 82.0      | 93.6      | 79.0       |             | 93.2     |          | 65.1   | 75.5   |
| VP1 (3477, 1158) | 76.7      | 96.8      | 71.3       |             |          |          | 64.4   | 67.0   |
| VP2 (2814, 937) | 77.5      | 97.6      | 72.6       |             | 72.8     |          | 68.5   | 68.5   |
| VP3 (2292, 763) | 94.5      | 94.9      | 94.3       |             | 94.9     |          | 64.8   | 64.8   |
| NSP1-1 (306, 101) | 76.7      | 97.5      | 72.3       |             | 97.0     |          | 61.2   | 66.5   |
| NSP1-2 (963, 320) | 71.0      | 96.7      | 72.3       |             | 93.5     |          | 53.0   | 57.3   |
| NSP2 (903, 300) | 80.4      | 92.6      | 76.7       |             | 92.0     |          | 68.3   | 68.3   |
| NSP3 (891, 296) | 94.4      | 95.2      | 95.3       |             | 97.0     |          | 48.6   | 48.6   |
| NSP4 (627, 208) | 93.0      | 95.2      | 94.2       |             |          |          | 47.3   | 47.3   |
| NSP5 (501, 166) | 78.6      | 95.6      | 85.0       |             | 98.8     |          | 49.8   | 49.8   |

The length of coding sequence nucleotides and amino acids are listed in parentheses.
The genome of the orf virus could not be assembled due to the limited number of reads. Orf virus is an epitheliotropic virus, which causes pustular dermatitis (contagious ecthyma or ‘sore mouth’) in goats and sheep, and is capable of infecting multiple ruminant and mammalian species [32]. These goat kids lacked skin or oral lesions, and thus, detection of this virus is evidence of environmental exposure or possibly an acute subclinical infection, but it is unrelated to enteritis in these goat kids.

The RVB reads were de novo assembled, and the RVB gene segments were deposited into GenBank (KY689687 to KY689697) as RVB/Goat-wt/Minnesota-1/USA/2016. Complete genome characterization is essential to investigate the evolution of RVs as RVs have a natural ability to reassort during coinfections. The sequence alignment for each of the RVB gene segments was constructed using ClustalW in MEGA v.6 software with the available RVB sequences in GenBank. The nucleotide and amino acid sequence identities were analysed with the Lasergene package MegAlign software v7.1.0 (DNASTAR) (Table 1). The coding sequence length for all the gene segments of the caprine strain was the same as those of the bovine, porcine, human and rat RVB strains, except for the NSP3 gene, in which the caprine strain was 153 nt shorter than the human strains and 207 nt shorter than the rat strain. The caprine RVB strain shared the highest percentage nucleotide and amino acid identities with bovine RVB strains (71.0–98.1 % and 71.3–98.1 %, respectively) while the nucleotide and amino acid sequence identities of caprine RVB genes ranged from 43.3 to 77.6 % and 34.4 to 72.6 %, respectively, compared with those of porcine, human and rat RVB gene segments (Table 1).

Partial bovine RVB genomes have been reported from Japan and India (Table 2). The caprine VP7, VP6, VP2, NSP1, NSP2 and NSP5 gene segments had a higher percentage nucleotide identity to the cognate Japanese bovine RVB gene segments than to the Indian bovine RVB gene segments, except in the VP4 gene segment, which illustrates a Japanese bovine RVB genome constellation, with a Japanese VP4 gene segment.

Rotavirus Classification Working Group (RCWG) recommendations were developed and widely applied in RVA classification, but classification for RVB has not been developed for the 11 gene segments [33]. Recently, the nucleotide cut-off values of 80, 81, 70, 78 and 70 % were used to genotype the VP7, VP6, VP3, NSP3 and NSP4 gene segments of RVB strains, respectively [13, 16, 34]. The NSP1 and NSP5 gene of RVB were also divided into seven and six clusters [13], respectively. The classification methodology is hindered due to the limited number RVB strain sequences.

Phylogenetic trees were reconstructed using the maximum-likelihood algorithm, with the GTR nucleotide substitution model (bootstrap analysis with 1000 replicates) with MEGA v.6 software. In the phylogenetic trees for each of the 11 gene segments (Fig. 2a–k), the caprine RVB strain shared a common ancestor with the bovine RVB strains from Japan,
Fig. 2. Phylogenetic trees of different RVB gene segments, VP7 (a), VP4 (b), VP6 (c), VP1 (d), VP2 (e), VP3 (f), NSP1 (g), NSP2 (h), NSP3 (i), NSP4 (j) and NSP5 (k), of the study strain with cognate gene segments of the other RVB strains. Red circles indicate the different segments of the USA RVB strain: RVB/Goat-wt/USA/Minnesota-1/2016. The ovine, rat, bovine, pig and human rotavirus strains are highlighted in colours maroon, black, fuchsia, blue and green, respectively. Bars, 0.05 nucleotide substitutions per site.
except in the VP4 gene segment, which shared a common ancestor with the bovine RVB strains from India. These phylogenetic analyses were consistent with the above nucleotide identity analyses, suggesting an inter-species transmission between caprine and bovine RVB strains. Further studies are needed to understand the genetic and
interspecies relationships between caprine and bovine RVB strains.

In conclusion, this study identified RVB as the causative agent of enteritis in goat kids through the detection of rotavirus-like virions in the faeces of goat kid 2 and confirmation with positive RVB PCR of the intestinal contents. The lack of detection of virions in the faeces of goat kid 1 may indicate that the infection was in the pre-latent phase or that the amount of virus being shed in the faeces was below the threshold of detection by negative stain electron microscopy. The significance of the *Clostridium perfringens* isolated from the intestine of goat kid 1 is uncertain as this bacterium may be isolated from non-diarrhoeic goats. In addition, the complete caprine RVB genome was characterized using metagenomic NGS. The nucleotide sequences and phylogenetic analyses suggested the possible interspecies transmission between caprine and bovine RVB strains. Further studies should screen RVB in caprine and bovine herds to understand this evolutionary relationship within ruminants.

Funding information
The study was supported by the University of Minnesota Veterinary Diagnostic Laboratory (UMVDL). Computing resources were supported by the University of Minnesota Supercomputing Institute.

Acknowledgements
The authors thank the faculty and personnel at the UMVDL for their technical services. Fangzhou Chen was supported by the China Agriculture Research System (no. CARS-36) and China Scholarship Council no. 20166760012.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References
1. Estes M, Greenberg H. Rotaviruses. In: Knipe DM and Howley PM (editors), *Fields Virology*, 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013. pp. 1347–1401.
2. McDonald SM, Patton JT. Assortment and packaging of the segmented rotavirus genome. *Trends Microbiol* 2011;19:136–144.
3. Mathijssens J, Otto PH, Ciaret M, Desselberger U, van Ranst M et al. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. *Arch Virol* 2012;157:1177–1182.
4. Mihalov-Kovács E, Gellért Á, Marton S, Farkas SL, Fehér E et al. Candidate new rotavirus species in sheltered dogs, Hungary, *Emerg Infect Dis* 2015;21:660–663.
5. Bányai K, Kemenesi G, Budinski I, Földes F, Zana B et al. Candidate new rotavirus species in Schreiber’s bats, Serbia. *Infect Genet Evol* 2017;48:19–26.
6. Hung T, Chen GM, Wang CG, Yao HL, Fang ZY et al. Waterborne outbreak of rotavirus diarrhoea in adults in China caused by a novel rotavirus. *Lancet* 1984;1:1139–1142.
7. Shen S, McKee TA, Wang ZD, Desselberger U, Liu DX. Sequence analysis and in vitro expression of genes 6 and 11 of an ovine group B rotavirus isolate, KB63: evidence for a non-defective, C-terminally truncated NSP1 and a phosphorylated NSP5. *J Gen Virol* 1999;80:2077–2086.
8. Vonderfecht SL, Lindsay DA, Eiden JJ. Detection of rat, porcine, and bovine group B rotaviruses in fecal specimens by solid-phase enzyme immunoassay. *J Clin Microbiol* 1994;32:1107–1108.
9. Sanekata T, Ahmed MU, Kader A, Taniguchi K, Kobayashi N. Human group B rotavirus infections cause severe diarrhea in children and adults in Bangladesh. *J Clin Microbiol* 2003;41:2187–2190.
10. Sigolo de San Juan C, Bellinzoni RC, Mattion N, La Torre J, Scodeller EA. Incidence of group A and atypical rotaviruses in Brazilian pig herds. *Res Vet Sci* 1986;41:270–272.
11. Smitalova R, Rodak L, Smid B, Piskal I. Detection of nongroup A rotaviruses in faecal samples of pigs in the Czech Republic. *Veterinarni Medicina* 2009;54:12–18.
12. Lahon A, Walimbe AM, Chitambar SD. Full genome analysis of group B rotaviruses from western India: genetic relatedness and evolution. *J Gen Virol* 2012;93:2252–2266.
13. Hayashi-Miyamoto M, Murakami T, Minami-Fukuda F, Tsuchiaka S, Kishimoto M et al. Diversity in VP3, NSP3, and NSP4 of rotavirus B detected from Japanese cattle. *Infection, Genetics and Evolution* 2017.
14. Fu ZF, Blackmore DK, Hampson DJ, Wilks CR. Epidemiology of typical and atypical rotavirus infections in New Zealand pigs. *N Z Vet J* 1989;37:102–106.
15. Muñoz M, Alvarez M, Lanza I, Cármenes P. Role of enteric pathogens in the aetiology of neonatal diarrhoea in lambs and goat kids in Spain. *Epidemiol Infect* 1996;117:203–211.
16. Marthaler D, Rossow K, Kramer M, Collins J, Goyal S et al. Detection of substantial porcine group B rotavirus genetic diversity in the United States, resulting in a modified classification proposal for G genotypes. *Virology* 2012;433:85–96.
17. Brown DW, Beards GM, Chen GM, Flewett TH. Prevalence of antibody to group B (atypical) rotavirus in humans and animals. *J Clin Microbiol* 1987;25:316–319.
18. Ahmed MU, Kobayashi N, Wakuda M, Sanekata T, Taniguchi K et al. Genetic analysis of group B human rotaviruses detected in Bangladesh in 2000 and 2001. *J Med Virol* 2004;72:149–155.
19. Chitambar SD, Lahon A, Tatte VS, Maniya NH, Tambe GU et al. Occurrence of group B rotavirus infections in the outbreaks of acute gastroenteritis from western India. *Indian J Med Res* 2011;134:399–400.
20. Do LP, Doan YH, Nakagomi T, Gauchan P, Kaneko M et al. Whole genome analysis of Vietnamese G2P4 rotavirus strains possessing the NSP2 gene sharing an ancestral sequence with Chinese sheep and goat rotavirus strains. *Microbiol Immunol* 2015;59:606–613.
21. Ghosh S, Alam MM, Ahmed MU, Talukder RI, Paul SK et al. Complete genome characterization of a caprine group A rotavirus strain reveals common evolution with ruminant and human rotavirus strains. *J Gen Virol* 2010;91:2367–2373.
22. Louge Uriarte EL, Badaracco A, Mathijssens J, Zeller M, Heylen E et al. The first caprine rotavirus detected in Argentina displays genomic features resembling virus strains infecting members of the *Bovidae* and *Camelidae*. *Vet Microbiol* 2014;171:189–197.
23. Aziz MA. Present status of the world goat populations and their productivity. *World* 2010;86:11.
24. Smith MC, Sherman DM. Fundamentals of goat practice. *Goat Medicine*. Ames: Wiley-Blackwell; 2009. pp. 3–21.
25. Dharma K, Chauhan RS, Mahendran M, Malik SV. Rotavirus diarrhoea in bovines and other domestic animals. *Vet Res Commun* 2009;33:1–23.
26. Gueguen C, Maga A, McCrae MA, Bataillon G. Caprine and bovine B rotaviruses in western France: group identification by Northern hybridization. *Vet Res* 1996;27:171–176.
27. Marthaler D, Jiang Y, Collins J, Rossow K. Complete genome sequence of strain SDCV/USA/Illinois121/2014, a Porcine Delta-coronavirus from the United States. *Genome Announc* 2014;2:e00218-14.
28. Peng Y, Leung HC, Yiu SM, Chin FY. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics* 2012;28:1420–1428.
29. Tritt A, Eisen JA, Facciotti MT, Darling AE. An integrated pipeline for de novo assembly of microbial genomes. PLoS One 2012;7:e42304.

30. Knutson TP, Velayudhan BT, Marthaler DG. A porcine enterovirus G associated with enteric disease contains a novel papain-like cysteine protease. J Gen Virol 2017;98:1305–1310.

31. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods 2012;9:357–359.

32. Bergqvist C, Kurban M, Abbas O. Orf virus infection. Rev Med Virol 2017;27:e1932.

33. Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T et al. 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. J Virol 2008;82:3204–3219.

34. Marthaler D, Suzuki T, Rossov K, Culhane M, Collins J et al. VP6 genetic diversity, reassortment, intragenic recombination and classification of rotavirus B in American and Japanese pigs. Vet Microbiol 2014;172:359–366.

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