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Short communication

High detection rate and high genetic diversity of genogroup I Picobirnaviruses from roe deer

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ABSTRACT

Picobirnaviruses (PBVs) have been characterized as opportunistic enteric pathogens detected in various domestic, zoo and wild animals, suggesting a wide host range of these viruses. It is thus important to monitor wild animals for the presence of various human and animal pathogens in order to identify a potential reservoir of infectious diseases. In this study, the first phylogenetic analysis of PBV from roe deer (*Capreolus capreolus*) was performed with a total of 70 investigated samples of feces from roe deer collected in 2014 and 2015 during a survey throughout Slovenia. A high detection rate of PBVs was observed with newly designed specific primers, 42 samples out of 70 (60%) being positive. Phylogenetic analysis of the partial RdRp gene showed that roe deer PBV sequences were distributed over the whole phylogenetic tree and were distributed between 7 highly supported groups and 12 separate branches within the PBV genogroup I. The animal PBV strain most closely related to roe deer PBV strains was the Rhesus macaque PBV/BGD/PbV-55 strain, with 89.1% nucleotide identity to that of PBV SLO/D80-14. Overall nucleotide sequence identity between PBV strains obtained from roe deer ranged from 60.4 to 100%, confirming the high genetic diversity with no subtypes related to host species or geographic location in general. This first phylogenetic survey of roe deer PBVs provides further knowledge concerning the putative host range and confirms the high genetic diversity of these PBVs.

1. Introduction

Picobirnaviruses (PBVs) are small, non-enveloped viruses containing a bi-segmented, double-stranded (ds) RNA genome of around 4 kb. The larger genome segment 1 (2.2–2.7 kb) has from two to three open reading frames (ORFs), the largest ORF coding for CA protein. The smaller genome segment 2 (1.2–1.9 kb) has one ORF coding for the viral RNA-dependent RNA polymerase (RdRp) (Ganesh et al., 2014; Malik et al., 2014). Taxonomically, the *Picobirnavirus* genus is the sole member of the *Picobirnaviridae* family. PBVs have been classified into at least three genogroups, designated as GI, GII and GIII, based on differences in genome segment 2 (Delmas et al., 2019). At the present time, the majority of the molecularly characterized PBVs belong to the GI genogroup (Ganesh et al., 2014; Ghosh et al., 2018; Li et al., 2015; Luo et al., 2018; Malik et al., 2014; Navarro et al., 2018; Smits et al., 2014).

PBV was first detected in 1988, during an outbreak of acute gastroenteritis in Brazil, when the virus was detected in humans and black-footed pigmy rice rats (Pereira et al., 1988). Since then, it has been characterized as an opportunistic enteric pathogen and detected in various domestic, zoological and wild animals, suggesting a wide host range for this virus (Conceição-Neto et al., 2016; Duraisamy et al., 2018; Gallagher et al., 2017; Ganesh et al., 2014; Ghosh et al., 2018; Kunz et al., 2018; Lojkić et al., 2016; Malik et al., 2014; Malik et al., 2018; Masachessi et al., 2015; Verma et al., 2015; Woo et al., 2016). Recently, it was even suggested (Krishnamurthy and Wang, 2018) that PBVs are prokaryotic RNA viruses.

The detection of PBVs in feces of numerous diarrheic and healthy humans as well as animals has increased concern concerning the public health aspects regarding the transmission of these viruses and their potential zoonotic transmission (Ganesh et al., 2014). Wildlife populations constitute a large and often unknown reservoir of infectious diseases (Chomel et al., 2007). It is thus important to monitor wild animals for the presence of different human and animal pathogens to identify a potential reservoir of infectious diseases.

During a survey throughout Slovenia, designed to screen some of the game animals as a potential source of enteric viruses, a PBV strain was detected in a sample of roe deer by next generation sequencing (NGS). The complete genome of this PBV strain was obtained and, to date, constitutes the only report of PBV detected from roe deer (Kuhar

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et al., 2017). The present study was undertaken to screen the feces of roe deer with newly designed specific primers, to determine the prevalence of PBV and to carry out phylogenetic analyses.

2. Materials and methods

2.1. Animal and sample collection

A total of 70 samples of feces from roe deer (*Capreolus capreolus*), collected in 2014 and 2015, were investigated and tested for the presence of PBVs. These samples were collected in the frame of a survey throughout Slovenia in which certain game animals, including roe deer, were screened for their potential as a source of enteric viruses. The sample collection, preparation of suspensions and virus RNA extraction was previously described (Jamnikar-Ciglenecki et al., 2016).

2.2. Design of specific primers, RT-PCR and nucleotide sequencing

Sequence analysis of segment 2 of the PBV/roe_deer/SLO/D38-14/2014 strain obtained by Kuhar et al. (2017) with NGS (GenBank accession number MG190029) led to the suggestion that the most frequently used primers for detecting PBV genogroup I strains with RT-PCR, designed by Rosen et al. (2000) could be suboptimal for detecting the roe deer PBVs. New primers, the PBV F1143 (5′–AACCCAAATTCA CAGTGTCTTGG–3′) and the PBV R1468 (5′–AGAGGATGGTACTTCAC ATTCTC–3′) amplifying a 326 bp long product, were therefore designed with Primer3 (Untergasser et al., 2012) on the basis of aligned nucleotide (nt) sequences in the RdRp gene region from the aforementioned roe deer PBV strain and from PBV strains retrieved from GenBank. To be able to compare the PBV sequences from roe deer to not only the complete RdRp PBV sequences deposited in GenBank but also to partial RdRp PBV sequences deposited in GenBank, the primers were designed within the nucleotide alignment region where most of the PBV sequences retrieved from GenBank aligned. Amplification of a 326 bp long product with RT-PCR was performed using SuperScript One-Step RT-PCR with Platinum Taq (Invitrogen, CA, USA) in Mastercycler Nexus Gradient (Eppendorf, Germany). The final reaction volume of 25 μL was composed of 12.5 μL 2× reaction mix, 0.8 μL DNase/RNase-free water, 1 μL (0.2 pmol/μL) of each primer and 2 μL of RNA. Thermocycling conditions were 30 min at 50 °C and 2 min at 94 °C, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 58 °C for 30 s and extension at 72 °C for 1 min, ending with final extension at 72 °C for 10 min.

The PCR products were visualized with the QIAxcel Capillary Electrophoresis System (Qiagen, Germany) and subjected to direct Sanger sequencing with prior purification. Sequencing was performed by Macrogen Inc. (The Netherlands).

Sequences were deposited in GenBank under accession numbers MH516255-MH516284.

2.3. Phylogenetic analysis

Nucleotide sequences were assembled and edited with SeqMan and EditSeq software implemented in the DNASTAR program (Lasergene, WI, USA) and later compared with the PBV sequences deposited in GenBank using BLASTn. The phylogenetic analysis of the partial RdRp gene was performed. To assess the phylogenetic relationship of the roe deer PBV sequences with genogroup I PBV sequences deposited in GenBank, the initial phylogenetic analysis included sequences of genogroup I PBV strains retrieved from GenBank based on BLAST results, complete segment 2 genogroup I PBV sequences and also partial segment 2 genogroup I sequences within the matching region. For the final phylogenetic tree, only sequences relevant, in terms of phylogenetic relationship, to roe deer PBV sequences were selected and included in the analysis. The PBV strain Human PBV/USA/4-GA-91 (AF246940) belonging to genogroup II was used as an outgroup sequence. Some PBV nucleotide sequences investigated in this study were trimmed to 230 bp due to low quality bases at both ends, thus nucleotide alignments of 230 bp were used for the analysis. Nucleotide alignments were constructed using the MAFFT program (Katoh and Standley, 2013) and nucleotide identities were determined from the nucleotide alignment using Geneious software suite v 10.2.3 (Biomatters Ltd., New Zealand). The best fitting nucleotide substitution model was determined based on the lowest BIC scores. Phylogenetic trees were constructed with MEGA 7.0.21 (Kumar et al., 2016), using the ML method with the GTR + G + I substitution model. Statistical support for the trees was evaluated by bootstrapping, based on 1000 repetitions. Multiple alignments were created using the 31 PBV nt sequences of roe deer from Slovenia (30 sequences of the partial RdRp gene, together with the complete segment 2 sequence of PBV/roe_deer/SLO/D38-14/2014 strain) and selected sequences of PBV strains derived from GenBank. The complete segment 2 sequence of PBV/roe_deer/SLO/D38-14/2014 strain (MG190029) was previously published (Kuhar et al., 2017).

3. Results and discussion

In this study, a high detection rate of PBVs in samples of feces from roe deer and high genetic diversity of PBV strains in roe deer were observed. Of the 70 feces samples tested, 42 (60%) were positive for PBV with the RT-PCR. Nucleotide (nt) sequences were obtained from all the 42 PCR products but, due to nucleotide polymorphisms observed in 11 samples, only 31 nucleotide sequences were used for further phylogenetic analysis. The presence of nucleotide polymorphisms suggests that multiple strains of PBV are present in one sample, as described previously (Ribeiro Silva et al., 2014; Verma et al., 2015; Woo et al., 2016).

To investigate the genetic relationship between the roe deer PBV strains and PBV strains from other animals and humans, phylogenetic trees of a part of the RdRp gene were constructed and nt sequence identities calculated from multiple alignment. According to BLASTn results, the roe deer PBV strains were most closely related to PBV genogroup I strains, so the selected genogroup I PBV strains from GenBank were included in the phylogenetic analysis.

On the phylogenetic tree, the PBV strains detected from roe deer were distributed between 7 highly supported groups (with bootstrap values > 80% and nucleotide identity > 90%) and 12 separate branches within PBV genogroup I (Fig. 1). Overall nt sequence identity between the 31 PBV strains obtained from roe deer ranged from 60.4 to 100%.

According to the phylogenetic tree and to nt identities, some of the roe deer PBV strains clustered with other animal and human PBV strains with high statistical support. The animal PBV strain most closely related to roe deer PBV strains was the Rhesus macaque PBV/BGD/PbV-55 strain with 89.1% nt identity compared to PBV SLO/D80-14. The PBV SLO/D36-14 clustered with human, gorilla and macaque PBV strains (PBV/BEL/HPBV1352/2010, PBV/USA/Pak-HPBV-2, PBV/IND/GPBV12, PBV/COG/2015 and PBV/USA/WUSTL-26/201) with nt identities from 86.5 to 87.8%. The roe deer PBV SLO/D2-14 and PBV SLO/D116-15 clustered with porcine (PBV/BEL/15V010/4815) and fox (PBV/NLD/F9) PBV strains with 81.7 to 86.5% nt identities. Two roe deer PBV strains (SLO/D15-14 and SLO/D56-14) clustered with genet strain PBV/ESP/S13 with 86.1 and 87% nt identity. Other animal and human PBV strains showed distant relationships to the roe deer PBV strains. According to a recent study of phylogenetic relationships of PBV sequences (Knox et al., 2018) in which a comparison of phylogenetic analysis of complete and trimmed PBV segment 2 lengths was performed, it is evident that the trimming of segment 2 sequences influences the clustering of these sequences. Thus, the roe deer PBVs might show different clustering with other animal PBVs, if a longer fragment of segment 2 was used for the phylogenetic analysis.

Seven highly supported groups of roe deer PBV sequences were observed on the phylogenetic tree. The PBV strains SLO/D15–14 and

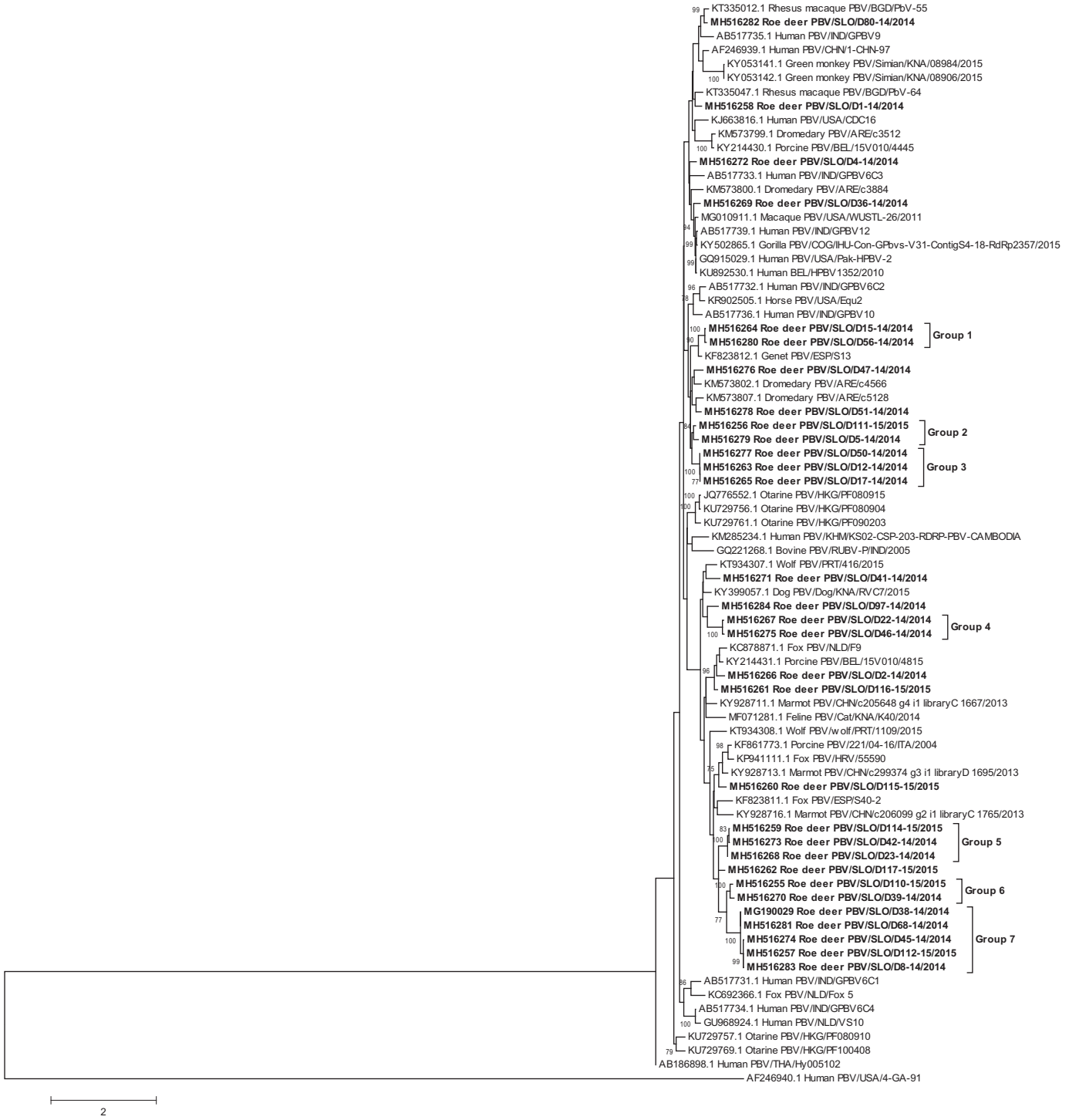

Fig. 1. The ML phylogenetic tree with the GTR + G + I substitution model of partial (230 bp) RdRp gene nucleotide sequences. Bootstrap values lower than 70 are not shown. The Slovenian roe deer PBV strains are bolded. Scale bar corresponds to 2 substitutions per nucleotide.

SLO/D56–14 formed the first group and shared 97% nucleotide identity between them. The second group was composed of two PBV strains (SLO/D11-15 and SLO/D5-14) with 90.4% nt identity. The three viral sequences (SLO/D50-14, SLO/D12-14 and SLO/D17-14) from the third group were from 99.6 to 100% identical. The fourth group included two PBV strains (SLO/D22-14 and SLO/D46-14) with 95.7% nt identity between them. Three PBV strains (SLO/D42-14, SLO/D114-15 and SLO/D23-14), exhibited from 97 to 98.3% nucleotide identity and formed the fifth group. The sixth group consisted of two PBV strains (SLO/D39-14 and SLO/D110-15) which shared 90.4% nt identity. Five PBV strains (SLO/D38-14, SLO/D68-14, SLO/D45-14, SLO/D8-14 and SLO/D112-15) formed the seventh group showing from 94.8 to 99.6% nt identities. Within this group, there were two subgroups. One subgroup was composed of SLO/D38-14 and SLO/D68-14 PBV sequences that were 99.6% identical. The other subgroup was composed of SLO/D45-14, SLO/D8-14 and SLO/D112-15 PBV sequences which were from 98.7 to 99.6% identical. High nucleotide identity of sequences from the third group and the two subgroups within the seventh group suggests that these animals might be infected with the same PBV strain, respectively. On the phylogenetic tree, the 12 PBV strains obtained from

roe deer (SLO/D80-14, SLO/D1-14, SLO/D4-14, SLO/D36-14, SLO/D47-14, SLO/D51-14, SLO/D97-14, SLO/D2-14, SLO/D116-15, SLO/D115-15, SLO/D117-15 and SLO/D41-14), were placed on 12 branches between other roe deer and other animals, as well as human PBV strains. The nucleotide identities of these 12 PBV strains were from 77.4% to 89.1%, compared to their most closely related PBV strains.

The majority of PBVs identified worldwide belong to the genogroup I (Ganesh et al., 2014; Malik et al., 2014). According to observations from our study, as well as from several other studies regarding the PBV genetic diversity, the genogroup I PBV observed worldwide show high genetic diversity with no subtypes related to host species or geographic location in general (Kunz et al., 2018; Malik et al., 2018; Ribeiro Silva et al., 2014; Woo et al., 2014). Phylogenetic analysis of the roe deer PBV strains showed that these sequences were distributed over the whole phylogenetic tree (Fig. 1), which was also observed in other studies of animal PBVs (Verma et al., 2015; Woo et al., 2016). Some reports describe phylogenetic clusters of closely related PBV sequences from the same host species originating from the same geographic location (Gallagher et al., 2017; Ribeiro Silva et al., 2014; Verma et al., 2015; Woo et al., 2016). Several studies also describe closely related PBV strains originating from different species, from different geographical locations and at different times (Anthony et al., 2015; Bányai et al., 2008; Duraisamy et al., 2018; Ribeiro Silva et al., 2014). In this study, clustering of closely related roe deer PBV strains and also clustering of roe deer with other animal and human PBV strains was observed.

Phylogenetic analyses of genogroup I PBV strains from this and other studies suggest that PBVs infect a variety of host species worldwide, are able to transmit between species and are not host specific (Duraisamy et al., 2018; Gallagher et al., 2017; Ganesh et al., 2014; Ghosh et al., 2018; Luo et al., 2018; Malik et al., 2018; Verma et al., 2015; Woo et al., 2016). Based on the fact that PBVs are detected in human and animal feces samples, it is anticipated that these viruses infect animal or human host. However, a recent comparative genomic study of PBV sequences (Krishnamurthy and Wang, 2018) suggested, that PBVs are in fact RNA viruses that infect prokaryotes.

In conclusion, this first phylogenetic survey of roe deer PBV provides new knowledge and understanding of the putative host range and confirms the high genetic diversity of PBVs.

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References

Anthony, S.J., Islam, A., Johnson, C., Navarrete-Macias, I., Liang, E., Jain, K., Hitchens, P.L., Che, X., Soloyvov, A., Hicks, A.L., Ojeda-Flores, R., Zambrana-Torrelio, C., Ulrich, W., Rostal, M.K., Petrosov, A., Garcia, J., Haider, N., Wolfe, N., Goldstein, T., Morse, S.S., Rahman, M., Epstein, J.H., Mazet, J.K., Daszak, P., Lipkin, W.I., 2015. Non-random patterns in viral diversity. Nat. Commun. 6, 8147.

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

Chomel, B.B., Belotto, A., Meslin, F.X., 2007. Wildlife, exotic pets, and emerging zoonoses. Emerg. Infect. Dis. 13, 6–11.

Conceição-Neto, N., Mesquita, J.R., Zeller, M., Yinda, C.K., Álvares, F., Roque, S., Petrucci-Fonseca, F., Godinho, R., Heylen, E., Van Ranst, M., Matthijnssens, J., 2016. Reassortment among Picobirnaviruses found in wolves. Arch. Virol. 161, 2859–2862.

Delmas, B., Attoui, H., Ghosh, S., Malik, Y.S., Mundt, E., Vakharia, V.N., Ictv Report Consortium, 2019. ICTV virus taxonomy profile: Picobirnaviridae. J. Gen. Virol. 100, 133–134.

Duraisamy, R., Akiana, J., Davoust, B., Mediannikov, O., Michelle, C., Robert, C., Parra, H.J., Raoult, D., Biagini, P., Desnues, C., 2018. Detection of novel RNA viruses from free-living gorillas, Republic of the Congo: genetic diversity of Picobirnaviruses. Virus Genes 54, 256–271.

Gallagher, C.A., Navarro, R., Cruz, K., Aung, M.S., Ng, A., Bajak, E., Beierschmitt, A., Lawrence, M., Dore, K.M., Ketzis, J., Malik, Y.S., Kobayashi, N., Ghosh, S., 2017. Detection of picobirnaviruses in vervet monkeys (Chlorocebus sabaeus): molecular characterization of complete genomic segment-2. Virus Res. 230, 13–18.

Ganesh, B., Masachessi, G., Mladenova, Z., 2014. Animal picobirnavirus. Virusdisease 25, 223–238.

Ghosh, S., Shiokawa, K., Aung, M.S., Malik, Y.S., Kobayashi, N., 2018. High detection rates of picobirnaviruses in free roaming rats (Rattus spp.): molecular characterization of complete gene segment-2. Infect. Genet. Evol. 65, 131–135.

Jamnikar-Ciglenecki, U., Kuhar, U., Sturm, S., Kirbis, A., Racki, N., Steyer, A., 2016. The first detection and whole genome characterization of the G6P[15] group a rotavirus strain from roe deer. Vet. Microbiol. 191, 52–59.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Knox, M.A., Gedye, K.R., Hayman, D.T.S., 2018. The challenges of analysing highly diverse picobirnavirus sequence data. Viruses 10.

Krishnamurthy, S.R., Wang, D., 2018. Extensive conservation of prokaryotic ribosomal binding sites in known and novel picobirnaviruses. Virology 516, 108–114.

Kuhar, U., Vengust, G., Jamnikar-Ciglenecki, U., 2017. Complete genome sequence of Roe deer Picobirnavirus strain PBV/roe_deer/SLO/D38-14/2014. Genome Announc. 5 e01329–17.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Kunz, A.F., Possatti, F., de Freitas, J.A., Alfieri, A.A., Takiuchi, E., 2018. High detection rate and genetic diversity of picobirnavirus in a sheep flock in Brazil. Virus Res. 255, 10–13.

Li, L., Giannitti, F., Low, J., Keyes, C., Ullmann, L.S., Deng, X., Aleman, M., Pesavento, P.A., Pusterla, N., Delwart, E., 2015. Exploring the virome of diseased horses. J. Gen. Virol. 96, 2721–2733.

Lojkić, I., Biđin, M., Prpić, J., Šimić, I., Krešić, N., Bedeković, T., 2016. Faecal virome of red foxes from peri-urban areas. Comp. Immunol. Microbiol. Infect. Dis. 45, 10–15.

Luo, X.L., Lu, S., Jin, D., Yang, J., Wu, S.S., Xu, J., 2018. Marmota himalayana in the Qinghai-Tibetan plateau as a special host for bi-segmented and unsegmented picobirnaviruses. Emerg. Microbes Infect. 7, 20.

Malik, Y.S., Kumar, N., Sharma, K., Dhama, K., Shabbir, M.Z., Ganesh, B., Kobayashi, N., Banyai, K., 2014. Epidemiology, phylogeny, and evolution of emerging enteric Picobirnaviruses of animal origin and their relationship to human strains. Biomed. Res. Int. 2014, 780752.

Malik, Y.S., Sircar, S., Dhama, K., Singh, R., Ghosh, S., Bányai, K., Vlasova, A.N., Nadia, T., Singh, R.K., 2018. Molecular epidemiology and characterization of picobirnaviruses in small ruminant populations in India. Infect. Genet. Evol. 63, 39–42.

Masachessi, G., Ganesh, B., Martinez, L.C., Giordano, M.O., Barril, P.A., Isa, M.B., Paván, G.V., Mateos, C.A., Nates, S.V., 2015. Maintenance of picobirnavirus (PBV) infection in an adult orangutan (Pongo pygmaeus) and genetic diversity of excreted viral strains during a three-year period. Infect. Genet. Evol. 29, 196–202.

Navarro, J.O., Candido, M., de Almeida-Queiroz, S.R., Buzinaro, M.D.G., Livonesi, M.C., Fernandes, A.M., de Sousa, R.L.M., 2018. Genetic diversity of bovine Picobirnavirus, Brazil. Virus Genes 54 (5), 724–728.

Pereira, H.G., Flewett, T.H., Candeias, J.A., Barth, O.M., 1988. A virus with a bisegmented double-stranded RNA genome in rat (Oryzomys nigripes) intestines. J. Gen. Virol. 69 (Pt 11), 2749–2754.

Ribeiro Silva, R., Bezerra, D.A., Kaiano, J.H., Oliveira, D.e.S., Silvestre, R.V., Gabbay, Y.B., Ganesh, B., Mascarenhas, J.D., 2014. Genogroup I avian picobirnavirus detected in Brazilian broiler chickens: a molecular epidemiology study. J. Gen. Virol. 95, 117–122.

Rosen, B.I., Fang, Z.Y., Glass, R.I., Monroe, S.S., 2000. Cloning of human picobirnavirus genomic segments and development of an RT-PCR detection assay. Virology 277, 316–329.

Smits, S.L., Schapendonk, C.M., van Beek, J., Vennema, H., Schürch, A.C., Schipper, D., Bodewes, R., Haagmans, B.L., Osterhaus, A.D., Koopmans, M.P., 2014. New viruses in idiopathic human diarrhea cases, the Netherlands. Emerg. Infect. Dis. 20, 1218–1222.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S.G., 2012. Primer3—new capabilities and interfaces. Nucleic Acids Res. 40, e115.

Verma, H., Mor, S.K., Erber, J., Goyal, S.M., 2015. Prevalence and complete genome characterization of Turkey picobirnaviruses. Infect. Genet. Evol. 30, 134–139.

Woo, P.C., Lau, S.K., Teng, J.L., Tsang, A.K., Joseph, M., Wong, E.Y., Tang, Y., Sivakumar, S., Bai, R., Wernery, R., Wernery, U., Yuen, K.Y., 2014. Metagenomic analysis of viromes of dromedary camel fecal samples reveals large number and high diversity of circoviruses and picobirnaviruses. Virology 471-473, 117–125.

Woo, P.C., Teng, J.L., Bai, R., Wong, A.Y., Martelli, P., Hui, S.W., Tsang, A.K., Lau, C.C., Ahmed, S.S., Yip, C.C., Choi, G.K., Li, K.S., Lam, C.S., Lau, S.K., Yuen, K.Y., 2016. High diversity of Genogroup I Picobirnaviruses in mammals. Front. Microbiol. 7, 1886.