Arch Virol (2016) 161:177–180
DOI 10.1007/s00705-015-2638-1

BRIEF REPORT

First detection of ungulate tetraparvovirus 1 (bovine hokovirus 1) in domestic yaks in northwestern China

Fang Xu[1] · Yangyang Pan[1] · Meng Wang[2] · Xin Wu[1] · Lili Tian[3] · Abdul Rasheed Baloch[4] · Qiaoying Zeng[1]

Received: 9 July 2015 / Accepted: 4 October 2015 / Published online: 19 October 2015

Abstract We describe the discovery and phylogenetic analysis of ungulate tetraparvovirus 1 (also referred to as bovine hokovirus 1, B-PARV4, or partetravirus) in domestic yaks (*Bos grunniens*) in northwestern China. The yak B-PARV4 genome was detected in yak blood samples by PCR, using B-PARV4 primers corresponding to conserved regions. Twenty-two of 370 samples were positive for a B-PARV4-related genome sequence, indicating an overall prevalence of 5.95 %. The prevalence in Qinghai Province (13/195, 6.67 %) and Gansu Province (9/175, 5.14 %) was similar, but it varied significantly between yaks ≤ 1 year old (15/177, 8.47 %) and yaks > 1 year old (7/193, 3.6 %) ($p < 0.05$). An alignment of the nearly full-length genome sequences of all 22 strains identified six different genomic sequences. A phylogenetic analysis revealed 99.0–99.7 % sequence identity between these six genomes and all known B-PARV4 genomes, excluding JF504698 (only 88.6 % identity), which represents another genotype. This is the first discovery of B-PARV4-related viruses in domestic yaks.

F. Xu and Y. Pan contributed equally to this article.

✉ Qiaoying Zeng
zengqy@gsau.edu.cn

Yangyang Pan
panyangyang_2007@126.com

[1] The College of Veterinary Medicine, Gansu Agricultural University, Lanzhou, People's Republic of China

[2] The College of Veterinary Medicine, Northwest A&F University, Yangling, People's Republic of China

[3] China Animal Health and Epidemiology Center, Qingdao, People's Republic of China

[4] Faculty of Veterinary Science, Sindh Agriculture University, Tandojam, Pakistan

Introduction

Tetraparvovirus (previously proposed name, "*Partetravirus*") is a newly established genus in the family *Parvoviridae* [1, 2] that includes primate tetraparvovirus 1 (human parvovirus 4, PARV4) and five novel PARV4-related animal parvoviruses described in the last decade: chiropteran tetraparvovirus 1 (*Eidolon helvum* [bat] parvovirus, Ba-PARV4), chimpanzee parvovirus 4 (Ch-PARV4), ungulate tetraparvovirus 1 (bovine hokovirus 1, B-PARV4), ungulate tetraparvovirus 2 (porcine hokovirus, P-PARV4), ungulate tetraparvovirus 3 (porcine Cn virus, CnP-PARV4), and ungulate tetraparvovirus 4 (ovine hokovirus, O-PARV4). Members of the family *Parvoviridae* are small, non-enveloped, single-stranded DNA viruses with an icosahedral capsid. They infect a wide range of hosts and are divided into two subfamilies: *Parvovirinae*, whose members infect vertebrates, and *Densovirinae*, whose members infect insects and other arthropods [1, 2]. Many novel parvoviruses have been discovered in recent years, although, historically, they were considered genetically stable [1–8]. Therefore, the subfamily *Parvovirinae* has been expanded to include, in addition to the previous five genera, three new genera, *Aveparvovirus* (*Ave*, bird), *Copiparvoviru* (*Copi*, cow and pig) and *Tetraparvovirus* [1, 2]. There have been only two reports describing members of the species *Ungulate tetraparvovirus 1*, B-PARV4-1 and B-PARV4-2, which were discovered in Hong Kong by the same research group in 2008 and 2011, respectively [3, 4]. To date, there has been no other report of this novel parvovirus anywhere in the world.

Since their domestication at least 5000 years ago by ancestors of the present Chinese Tibetan people, yaks (*Bos grunniens*) have lived exclusively in the cold highlands surrounding the Qinghai-Tibet Plateau (altitudes > 3000 m,

average annual temperature < 0 °C), including Qinghai and Gansu Provinces in northwestern China. Because they live in a relatively secluded and very cold geographic region, yaks were never suspected of hosting any pathogens prior to recent discoveries of yaks carrying hepatitis E virus and *Brucella* spp. [9, 10]. We have been involved in research on P-PARV4 and the surveillance of yak infections [8, 9]. The aim of this study was to determine whether yaks host the recently discovered virus B-PARV4. We detected B-PARV4 in domestic yaks in China for the first time and determined its nearly full-length genomic sequence by PCR.

In this study, 370 blood samples (195 from Qinghai Province and 175 from Gansu Province) were collected from apparently healthy domestic yaks between May and October 2014 (Table 1). DNA was extracted from all of the samples, using an E-Z 96® Blood DNA Kit (Omega) according to the manufacturer's instructions, and it was used as the template for PCR amplification of full-length sequences. To detect PARV4-related sequences, the conserved primers B-PARV4-F and B-PARV4-R (Table 2) were designed, targeting a 629-bp fragment of the conserved VP2 region based on a multiple alignment of the reference PARV4 genomic sequences available in the National Center for Biotechnology Information (NCBI) GenBank database. Precautions were taken to avoid PCR contamination, and no false positives were permitted in the negative controls. All positive samples were subjected to nearly full-length sequencing. (Sequencing the full-length genome was hampered by the two terminal hairpin structures in the linear DNA genomes of parvoviruses.)

The nearly full-length genomic sequences were determined (two replicates for each sample) by multiplex PCR amplification using four pairs of primers (Table 2) that target overlapping fragments spanning the complete coding region of the PARV4 genome. The PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany), sequenced (TaKaRa, Dalian, China), assembled, and edited manually to produce the final sequences of the viral genomes. The sequence divergence of the genomes detected in this study was determined by multiple alignment and phylogenetic analysis of the genomic sequences of all of the B-PARV4s, O-PARV4s, and P-PARV4s available in the NCBI GenBank database, using the MEGA version 5 software and 1000 bootstrap replications.

PCR detected 22 positive samples among the 370 samples tested, with an overall prevalence of 5.95 %. The prevalence was similar in Qinghai (13/195, 6.67 %) and Gansu Provinces (9/175, 5.14 %) but varied significantly between the two age groups tested: 8.47 % (15/177) in yaks ≤ 1 year old and 3.6 % in yaks > 1 year old (7/193). The incidence in the younger group (15/22, 68.18 %) was more than twice that in the older group (7/22, 31.82 %) ($p < 0.05$; Table 1). Six different nearly full-length genomic sequences were identified using a multiple alignment of all of the genome sequences identified in the 22 positive samples, with no sequence discrepancies between the two replicates of each sample. The sequences were deposited in GenBank (accession numbers KT225725–KT225730), designated Yak/GS1–2 (two from Gansu Province) and Yak/QH1–4 (four from Qinghai Province). A phylogenetic tree clearly showed three main branches, encompassing B-PARV4s, O-PARV4s, and P-PARV4s. The six new yak genomes clustered on the same branch as all known B-PARV4s, including B-PARV4-1 (EU200669/bovine/HK1) and B-PARV4-2 (JF504698/bovine/HK4-B38) from Hong Kong, indicating that yaks host B-PARV4s, but not P-PARV4s or O-PARV4s. The new yak B-PARV4s shared 61.9–65.3 % and 64.7–68.3 % sequence identity with P-PARV4s and O-PARV4s, respectively, and 99.0–99.7 % identity with each other and other known B-PARV4s except JF504698/bovine/HK4-B38, with which they shared only 88.6 % sequence identity (Fig. 1).

On the phylogenetic tree, the main branch of B-PARV4s was divided into two further branches. JF504698/bovine/HK-B38 was the only strain on one branch [11], representing a separate genotype. The six new yak B-PARV4 strains were closely related to each other and formed one branch with EU200669/bovine/HK1, EU200668/bovine/HK3, and JF504697/bovine/HK-B15, demonstrating that only one B-PARV4 genotype is circulating in the yak populations in China.

The detection and phylogenetic analysis of this virus demonstrates for the first time the presence of B-PARV4s

Table 1 Frequency of B-PARV4s detected by PCR assay in blood from domestic yaks of different ages in two provinces of northwestern China

Location (province)	Age (Years)	Number of samples tested	B-PARV4 prevalence in domestic yaks
Qinghai	≤ 1	93	9 (9.68 %)
	≥ 1	102	4 (3.92 %)
Gansu	≤ 1	84	6 (7.14 %)
	≥ 1	91	3 (3.30 %)

Table 2 Primers used for detection and full-length genome amplification of B-PARV4

Primer name	Primer sequence	Location[a]	Length[b]
B-PARV4-F	5'-GTTGGCATTAGTGCTATTGTGA-3'	3717-4345	629
B-PARV4-R	5'-ACCAAAGGATTGATAGCATATC-3'		
B-PARV4-F1	5'-CGGTGACATCACTTCCGCTTA-3'	13-1370	1358
B-PARV4-R1	5'-TCTGTCATACGGCCTTCYTCC-3'		
B-PARV4-F2	5'-CTGCGAGTACKGGTAARACATT-3'	1223-2246	1024
B-PARV4-R2	5'-GGACTGAGTCACCTTCTTCTGAGA-3'		
B-PARV4-F3	5'-TCTTGGTGGAACTGGTAGGGT-3'	2146-3868	1723
B-PARV4-R3	5'-CCTGGCAAAGTGTCYTGATTATG-3'		
B-PARV4-F4	5'-CKGTTTCTGACAGTGCTAGCG-3'	3772-5123	1352
B-PARV4-R4	5'-AGAATCAGTCTCACACCAAGCAC-3'		

F, forward; R, reverse orientation; Y = C/T; K = G/T; R = A/G

[a] Nucleotide positions are according to the genomes of B-PARV4 HK-B1 (JF504697), HK-B3 (JF504698), bovine hokovirus 1 B-PARV4-1 (EU200669) and bovine hokovirus 2 B-PARV4-2 (JF504697)

[b] Amplicon size is given in base pairs

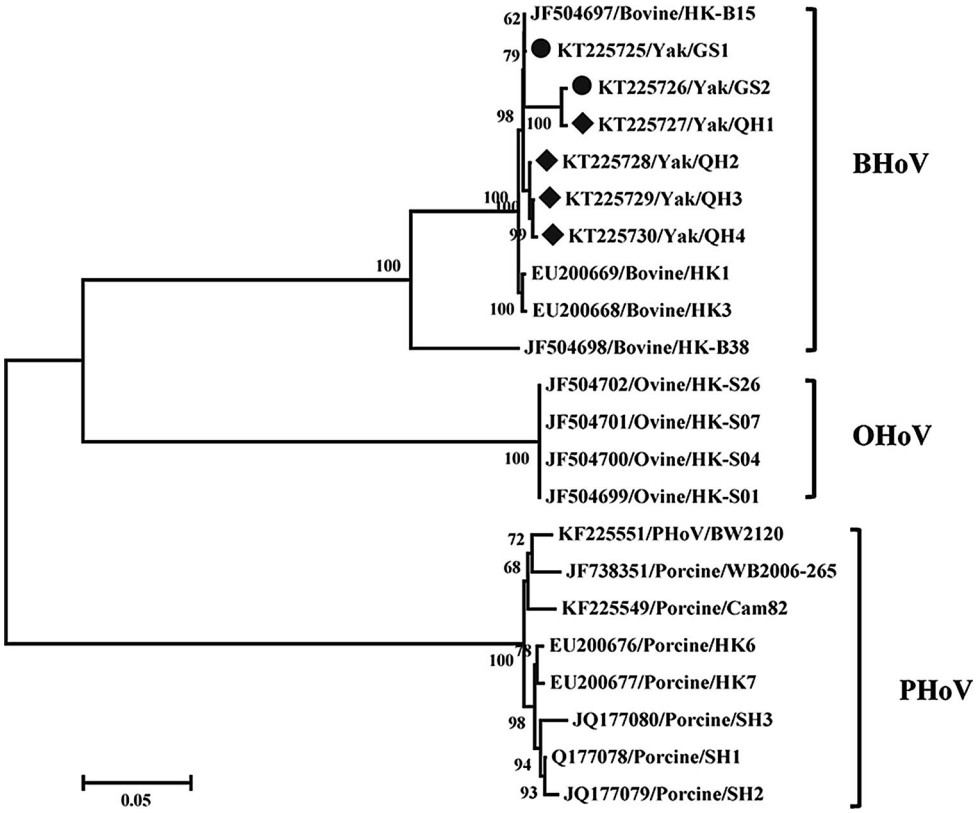

Fig. 1 Neighbor-joining tree showing the phylogenetic relationship among yak B-PARV4 strains based on nearly full-length gene sequences and comparison with previously identified bovine, porcine and ovine hokoviruses available in the GenBank database. Bootstrap values, expressed as percentages of 1,000 replications, are given at the branch points. GenBank accession numbers and animal species and strains are shown at each branch point. The two newly identified yak B-PARV4s strains in Gansu province described in the present study (GenBank accession numbers KT225725-KT225726) are indicated by ''●'', and four identified yak B-PARV4s strains in Qinghai province (GenBank accession numbers: KT225727-KT225730) are indicated by ''◆''. The scale bar indicates nucleotide substitutions per site

(but no other PARV4-related viruses) in a previously unsuspected host, domesticated highland yaks, extending our knowledge of the host range of B-PARV4. The yak B-PARV4s whose sequences are presented here are not associated with any disease, because the samples were collected from apparently healthy yaks. In previous studies of B-PARV4s, and also O-PARV4s and P-PARV4s, the viruses were identified in samples collected from both healthy and diseased animals, and no association was found with any disease when their prevalence was compared in the healthy and diseased animals. Therefore, the pathogenicity of B-PARV4s requires further detailed study. The overall prevalence (5.95 %) of yak B-PARV4 was much lower than that of B-PARV4s in bovines in Hong Kong (13 %) [3], and even lower than that of the closely related P-PARV4s in pigs, which generally ranges from

44.4 % to 71 % [3, 5–8], and that of O-PARV4s in ovine species (~70 %) [4]. The data for yak B-PARV4s reported here show a unique and significant negative correlation between prevalence and age, which is contrary to the phenomenon observed for PARV4 infections in other animals, such as B-PARV4 infections in bovine species, O-PARV4 infections in ovine species, and P-PARV4 infections in pigs [3–8].

Significantly, in this study, we have demonstrated the presence of B-PARV4s in yaks. There is as yet no sign of cross-species infection or transmission, and the detection and further analysis of B-PARV4 in wild yaks and other bovine species will be very important for clarifying the source, circulation pattern, zoonotic potential, and public-health risk of this virus. The mechanisms of its pathogenicity, transmission, evolution, and persistence also require urgent clarification.

Acknowledgments We thank China Animal Health and Epidemiology Center for their valuable assistance in sample collection. This work was supported by a grant from the National Natural Science Foundation of China (No. 31260616) and the Fuxi Foundation of Exceptional Talent at Gansu Agricultural University.

Compliance with ethical standards

Ethical statement All procedures in this study were approved by the Animal Care and Use Committee of Gansu Agricultural University and performed in accordance with animal welfare and ethics.

References

1. Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, Qiu J, Soderlund-Venermo M, Tattersall P, Tijssen P, Gatherer D (2014) The family Parvoviridae. Arch Virol 159:1239–1247
2. King AM, Adams MJ, Lefkowitz EJ (2011) Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier
3. Lau SK, Woo PC, Tse H, Fu CT, Au W-K, Chen X-C, Tsoi H-W, Tsang TH, Chan JS, Tsang DN (2008) Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. J Gen Virol 89:1840–1848
4. Tse H, Tsoi H-W, Teng JL, Chen X-C, Liu H, Zhou B, Zheng B-J, Woo PC, Lau SK, Yuen K-Y (2011) Discovery and genomic characterization of a novel ovine partetravirus and a new genotype of bovine partetravirus. PLoS One 6:25619
5. Szelei J, Liu K, Li Y, Fernandes S, Tijssen P (2010) Parvovirus 4-like virus in blood products. Emerg Infect Dis 16:561
6. Adlhoch C, Kaiser M, Ellerbrok H, Pauli G (2010) High prevalence of porcine Hokovirus in German wild boar populations. Virol J 7:1186
7. Cadar D, Cságola A, Lőrincz M, Tombácz K, Spînu M, Tuboly T (2011) Distribution and genetic diversity of porcine hokovirus in wild boars. Arch Virol 156:2233–2239
8. Pan Y, Zeng Q, Zhu C, Hua X, Wang M, Pan K, Cui L (2012) Frequency and characterization of porcine hokovirus (PHoV) in domestic pigs in eastern China. Arch Virol 157:1785–1788
9. Xu F, Pan Y, Baloch AR, Tian L, Wang M, Na W, Ding L, Zeng Q (2014) Hepatitis E virus genotype 4 in yak, northwestern china. Emerg Infect Dis 20:2182–2184
10. Zhu W, Dong JB, Zhang J, Uchida K, Watanabe K, Goto Y, Haga T (2013) Bos grunniens papillomavirus type 1: a novel deltapapillomavirus associated with fibropapilloma in yak. J Gen Virol 94:159–165
11. Lukashov VV, Goudsmit J (2001) Evolutionary relationships among parvoviruses: virus-host coevolution among autonomous primate parvoviruses and links between adeno-associated and avian parvoviruses. J Virol 75:2729–2740