AUSTRALIAN VETERINARY JOURNAL
AUSTRALIA'S PREMIER VETERINARY SCIENCE TEXT
SMALL ANIMALS

Genome sequence of an Australian strain of *canid alphaherpesvirus 1*

S Sarker,[a]* S Das,[b] K Helbig,[a] A Peters[b] and SR Raidal[b]

Objective Characterisation of a complete genome sequence of an Australian strain of *canid alphaherpesvirus 1* (CHV-1) and its phylogenetic relationship with other varicellovirus species.

Methods Standard pathology and PCR methods were used to initially detect herpesvirus in hepatic tissue from an infected 4-week-old Labrador Retriever puppy. The complete CHV-1 genome was sequenced using next-generation sequencing technology followed by de novo and reference assembly, and genome annotation.

Results The CHV-1 genome was 125 kbp in length and contained 74 predicted open reading frames encoding functional proteins, all of which have counterparts in other alphaherpesviruses. Phylogenetic analysis using the DNA polymerase gene revealed that the newly sequenced CHV-1 clustered with *canid alphaherpesvirus* isolated from the UK and shared a 99% overall nucleotide sequence similarity.

Conclusion This is the first complete genome of an Australian strain of CHV-1, which will contribute to our understanding of the genetics and evolution of herpesvirus.

Keywords acute hepatitis; dogs; herpesvirus; next-generation sequencing; whole genome

Abbreviations bp, base pairs; CHV-1, *canid alphaherpesvirus 1*; ORF, open reading frame

Aust Vet J 2018;96:24–27 doi: 10.1111/avj.12659

Canid alphaherpesvirus 1 (CHV-1; canine herpesvirus) is a well-known alphaherpesvirus that causes severe haemorrhagic disease in neonatal puppies worldwide. CHV-1 is a member of the genus *Varicellovirus* (subfamily *Alphaherpesvirinae*, Family *Alloherpesvirdae*, Order *Herpesvirales*), which has a double-stranded DNA (dsDNA) genome and has been a recognised cause of a fatal haemorrhagic syndrome of neonatal puppies since 1965.[1] The host range of CHV is generally restricted to domestic and wild *Canidae*[2] and the geographical distribution of CHV infection is varied, broadly ranging from 6% to 88% globally.[2,3] One study in 1971 demonstrated histological evidence of herpesvirus infection in puppies from Western Australia[4] and serological existence of CHV antibody has also been shown in the European red fox (*Vulpes vulpes*) in Australia.[5] Adult dogs infected with CHV are thought to not show any clinical signs, whereas puppies are more susceptible to CHV infection, leading to a generalised necrotising and haemorrhagic disease.[1] However, there is a lack of whole-genome genetic information relevant to canine herpesvirus infecting Australian dogs. To date, the complete genome of a canine herpesvirus from Australian puppies has not been described and therefore the phylogenetic reconstruction is not well-resolved. This study aimed to sequence and analyse the complete genome of CHV-1 from a clinical case of herpesvirus infection.

*Corresponding author.
[a]Department of Physiology, Anatomy and Microbiology, School of Life Sciences, La Trobe University, Bundoora, Victoria 3086, Australia; s.sarker@latrobe.edu.au
[b]School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, New South Wales, Australia

Materials and methods

Source of sampling

A 4-week-old female chocolate Labrador Retriever was presented to the Veterinary Diagnostic Laboratory at Charles Sturt University for necropsy examination. All three puppies in the litter died at ages 33, 34 and 35 days, respectively. Histological examination of hepatic tissue revealed widespread, multifocal coagulative necrosis of the hepatic parenchyma associated with haemorrhage and intranuclear eosinophilic inclusions. Similarly, there were multifocal areas of necrosis in the spleen, intestinal crypts, Peyer's patches and throughout the kidney.

Extraction of genomic DNA

A liver tissue sample (≈ 25 mg) was excised and chopped into small pieces that were aseptically transferred into a clean 1.5-mL microcentrifuge tube (Eppendorf) and genomic DNA was extracted using the Qiagen blood and tissue mini kit (Qiagen, Germany) before being stored at −20°C for until further testing. Initially, the extracted DNA was tested for herpesvirus by PCR using primers and conditions described for the amplification of the viral glycoprotein B gene.[6] Amplified PCR products, together with a standard molecular marker (Sigma-Aldrich, St Louis, MO, USA), were separated by electrophoresis using a 2.0% agarose gel and stained with GelRed (Biotium, CA, USA). Direct Sanger sequencing of the purified gel band confirmed the presence of herpesvirus (data not shown).

High-throughput sequencing

To obtain the entire sequence of the herpesvirus genome, next-generation sequencing (NGS) was used. DNA was extracted from stored liver tissue for NGS using QIAamp DNA mini (Qiagen) and unprotected nucleic acids were removed with the addition of 8 µL of RNase Cocktail Enzyme Mix (Invitrogen, San Diego, CA, USA). The genomic library was prepared with an insert size of 150-bp paired-end using the Illumina library preparation kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Whole-genome sequencing was performed on a HiSeq4000 sequencing platform (Illumina) by Novogene, China.

Bioinformatics

Assembly of the viral genome was conducted according to the established pipeline[3,7] in CLC Genomics workbench 9.5.3 (Qiagen,

Denmark) under the La Trobe University Genomics Platform. The HiSeq4000 generated 6,004,755 paired-end reads of 150 nucleotides. The preliminary quality evaluation for each raw read was generated using a quality control report. The raw data were pre-processed to remove ambiguous base calls, and bases or entire reads of poor quality using a probability limit of 0.5 %. Briefly, de novo assembly was performed and this generated large contigs with consensus sequences that corresponded strongly to the CHV sequence, according to BLASTp and BLASTn searches of GenBank databases.[8,9] These consensus sequences were used during reference assembly and CHV-1 (GenBank accession no. KT819633) was used as the reference herpesvirus.

The herpesvirus specific bioinformatics analyses were performed using the Viral Bioinformatics Resource Centre (www.virology.ca). The open reading frames (ORFs) longer than 50 codons with minimal overlap to other ORFs were predicted using Genome Annotation Transfer Utility (GATU)[10] and Geneious (version 10.0.2, Biomatters, NZ). These ORFs were subsequently extracted into a FASTA file and similarity searches, including nucleotide (BLASTn) and protein (BLASTp), were performed on annotated ORFs as potential genes if they shared significant sequence similarity to known viral or cellular genes (BLAST E value ≤ e-5) or contained a putative conserved domain as predicted by BLASTp.[11] The final CHV-1 annotation was further examined with other herpesvirus ortholog alignments to determine the correct methionine start site, correct stop codons, signs of truncation and validity of overlaps.

Phylogenetic analysis

Phylogenetic analysis was performed on translated protein sequences of the DNA polymerase of CHV-1 determined in this study, with closely related herpesvirus sequences. The amino acid sequences were aligned using the MAFFT L-INS-i algorithm (scoring matrix = BLOSUM62; gap open penalty = 1.53)[12] and the maximum likelihood tree under the LG substitution model, and 1000 non-parametric bootstrap resamplings, were generated using Geneious software.[13]

Results

Genome sequence and annotation of CHV-1

The CHV-1 whole-genome sequence was deposited in GenBank under the accession number KY057364. The final complete genome obtained from the Labrador puppy was 125,026 bp in length and exhibited an organisation typical of that of CHV. The overall nucleotide composition was 31.6% G + C, which is similar to other *canid alphaherpesvirus* sequences. The CHV-1 obtained in this study consists of a unique long sequence (97,320 bp) and a unique short

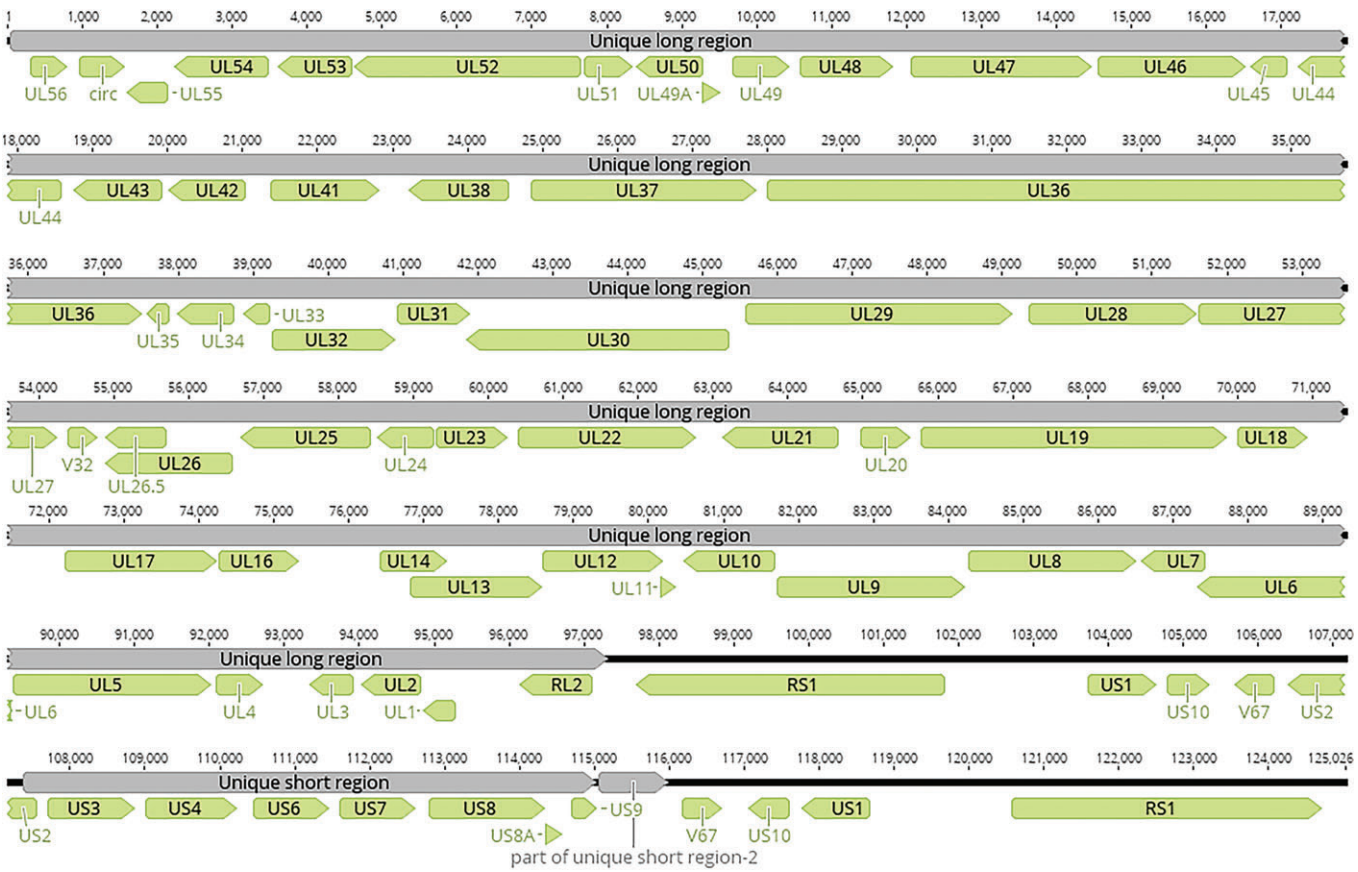

Figure 1. Schematic representation of the genome arrangement of *canid alphaherpesvirus 1*. Herpesviral conserved open reading frame are annotated in green using the CHV-UK ORF nomenclature. Unique long genome region and Unique short genome region marked in gray. CHV, canine herpesvirus.

sequence (8,645 bp) that are each flanked by terminal and internal inverted repeats (38 bp and 9,976 bp, respectively) (Figure 1). The genome contains 74 predicted ORFs encoding functional proteins, including DNA polymerase, envelope glycoprotein and transcriptional regulatory protein, all of which have counterparts in other members of the *Alphaherpesvirinae* subfamily (Supplementary Table 1). The functions and properties assigned to the predicted CHV-1 proteins determined in this study are based on the studies of their counterparts in other species of *Alphaherpesvirinae*.[14]

Phylogenetic analysis

Phylogenetic reconstruction from the translated protein sequences of the DNA polymerase gene (UL30) of CHV-1 (GenBank accession no. KY057364) from a puppy and other sequenced varicelloviruses showed it clustered with a CHV strain isolated from the UK (Figure 2). The Australian strain of CHV-1 sequence formed a well-supported clade (100%) with the CHV UK strain, indicating that it originated in this individual from a common ancestor.

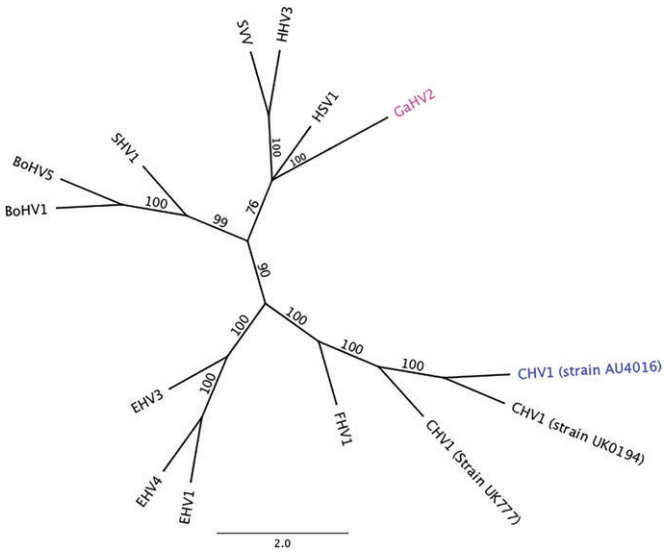

Figure 2. Phylogenetic relationship of varicellovirus DNA polymerase. The tree is rooted on gallid herpesvirus 2 (GaHV2, DQ530348) with 1000 bootstrap values shown for each branch. The abbreviations and GenBank accession details are: canid alphaherpesvirus 1 (CHV1-UK777, KT819633), canine herpesvirus 1 (CHV1-UK0194, NC_030117), canine herpesvirus 1 (CHV1-AU4016, KY057364), felid herpesvirus 1 (FHV1,-NC_013590), equine herpesvirus 1 (EHV1, NC_001491), equine herpesvirus 4 (EHV4, NC_001844), equine herpesvirus 3 (EHV3, NC_024771), bovine herpesvirus 1 (BoHV1, NC_001847), bovine herpesvirus 5 (BoHV5, NC_005261), Suid herpesvirus 1 (SHV1, NC_006151), human herpesvirus 3 (HHV3, NC_001348), simian varicella virus (SVV, NC_002686) and herpes simplex virus type 1 (HSV1, NC_001806).

Discussion

The main finding was the detection and characterisation of CHV-1 from a Labrador Retriever puppy, associated with the characteristic gross lesions of CHV disease in neonatal dogs. Immunologically naïve pregnant bitches are at risk of acute infection, which may be transmitted to fetuses or neonatal puppies, with the most significant systemic disease occurring in fetal or neonatal puppies from in utero infection, or infection in the first 3 weeks of life. In the case of this puppy, the dam was primiparous and aged 16 months when she had the litter.

The PCR testing and whole-genome analysis of DNA extracted from liver tissue confirmed the presence of CHV-1 and demonstrated strong similarity (> 99%) with previously published CHV isolated from dogs in the UK. The size of the genome (> 125 kb) determined in this study was consistent with other CHV genomes[3] and close to that which has been estimated by restriction endonuclease mapping of CHV strains.[15]

Our study confirmed one of the biological characteristics of varicelloviruses and other genera of the subfamily *Alphaherpesvirinae*, which contain genomes that are at the extremes of the G + C content range for all the herpesviruses.[3,15,16] The G + C content of the CHV-1 strain determined in this study (31.6%) was very close to genome sequences of UK CHV strains, which were isolated from archived samples stored for more than 10 years. Despite the great geographic and temporal separation it is somewhat surprising to have a high degree of similarity between the Australian CHV-1 genome and overseas CHV sequences. Nevertheless, this indicates that the CHV genome lacks genetic diversity, which is consistent with the description of the virus as monotypic.[17]

The phylogenetic relationship between CHV-1 and other varicelloviral DNA polymerase genes further confirmed the close relatedness of the CHV strain AU4016 with other herpesvirus strains. As shown in Figure 2, it is reasonable to postulate that these viruses originated from a common ancestor that diverged from a CHV-like progenitor, related to other species of varicellovirus with host-adaption over eons.

Conclusion

This is the first complete genome sequence of the CHV-1 Australian strain and was sequenced from a puppy with acute hepatic necrosis. We used high-throughput methods to determine the genome sequence of this CHV-1 strain, with sequence analysis demonstrating a remarkably high degree of similarity with other CHV-1 strains and providing a detailed gene map for the entire genome. The availability of this sequence will provide a unique approach to the understanding of the genetics and evolution of herpesvirus.

Acknowledgments

We gratefully acknowledge our microbiology technical staff, particularly Naomi Tidd and Michelle Ayton, for preserving the original samples, DNA extraction and screening.

Conflicts of interest and sources of funding

The authors declare no conflicts of interest or sources of funding for the work presented here.

References

1. Carmichael LE, Squire RA, Krook L. Clinical and pathologic features of a fatal viral disease of newborn pups. *Am J Vet Res* 1965;26:803–814.
2. Kawakami K, Ogawa H, Maeda K et al. Nosocomial outbreak of serious canine infectious tracheobronchitis (kennel cough) caused by canine herpesvirus infection. *J Clin Microbiol* 2010;48:1176–1181.
3. Papageorgiou KV, Suárez NM, Wilkie GS et al. Genome sequence of canine herpesvirus. *PLoS One* 2016;11:e0156015.
4. Nairn ME, Smith VW. Canine herpesvirus from a pup in Western Australia. *Aust Vet J* 1971;47:463–463.
5. Robinson AJ, Crerar SK, Waight Sharma N et al. Prevalence of serum antibodies to canine adenovirus and canine herpesvirus in the European red fox (*Vulpes vulpes*) in Australia. *Aust Vet J* 2005;83:356–361.
6. Burr PD, Campbell ME, Nicolson L et al. Detection of canine herpesvirus 1 in a wide range of tissues using the polymerase chain reaction. *Vet Microbiol* 1996;53:227–237.
7. Vaz PK, Mahony TJ, Hartley CA et al. The first genome sequence of a metatherian herpesvirus: Macropodid herpesvirus 1. *BMC Genomics* 2016;17:70.
8. Altschul SF, Gish W, Miller W et al. Basic local alignment search tool. *J Mol Biol* 1990;215:403–410.
9. Boratyn GM, Camacho C, Cooper PS et al. BLAST: a more efficient report with usability improvements. *Nucleic Acids Res* 2013;41:W29–W33.
10. Tcherepanov V, Ehlers A, Upton C. Genome Annotation Transfer Utility (GATU): rapid annotation of viral genomes using a closely related reference genome. *BMC Genomics* 2006;7:1–10.
11. Benson DA, Cavanaugh M, Clark K et al. GenBank. *Nucleic Acids Res* 2013;41:D36–42.
12. Katoh K, Misawa K, Kuma K et al. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 2002;30:3059–3066.
13. Guindon S, Gascuel O. A Simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 2003;52:696–704.
14. Davison AJ. Herpesvirus systematics. *Vet Microbiol* 2010;143:52–69.
15. Remond M, Sheldrick P, Lebreton F et al. Gene organization in the UL region and inverted repeats of the canine herpesvirus genome. *J Gen Virol* 1996;77:37–48.
16. Haanes EJ, Tomlinson CC. Genomic organization of the canine herpesvirus US region. *Virus Res* 1998;53:151–162.
17. Manning A, Buchan A, Skinner GR et al. The immunological relationship between canine herpesvirus and four other herpesviruses. *J Gen Virol* 1988;69:1601–1608.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://onlinelibrary.wiley.com/doi/10.1111/avj.12659/suppinfo.

Supplementary Table 1. Open reading frames of *canid alphaherpesvirus 1* (CHV-1)

(Accepted for publication 25 June 2017)