Characterization of a Novel Complete-Genome Sequence of a Galliform Chaphamaparvovirus from a Free-Range Laying Chicken Clinically Diagnosed with Spotty Liver Disease

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ABSTRACT This study reports a novel complete genome of galliform chaphamaparvovirus 4, which was detected in the bile of a free-range laying chicken diagnosed with spotty liver disease. The genome was 4,367 bp in length, enclosed by two identical inverted terminal repeats. The detection of this novel chaphamaparvovirus represents a notable concern for the poultry industry in Australia.

Chaphamaparvoviruses (ChPVs) are members of the family Parvoviridae and subfamily Hamaparvovirinae and are nonenveloped, icosahedral viruses with a linear single-stranded DNA genome of ~4.0 to 4.5 kb (1). They contain two major genes, namely, a nonstructural (NS) replicase gene and a capsid (VP) gene (2, 3). ChPVs are likely to be widespread in nature and have been detected in the feces of birds (4–8) and mammals (9), and a ChPV causes renal disease in laboratory mice (10). Recently, two novel ChPVs were detected in the livers of pheasants (Phasianus colchicus) (11) and in kidney tissue from a boobook owl (Ninox boobook) (12). Here, we report a novel complete genome of a ChPV, i.e., galliform ChPV 4 (GaChPV-4), that was detected in the bile of a free-range laying chicken that had been clinically diagnosed with spotty liver disease (SLD).

In 2021, a bile sample was collected from a chicken had been clinically diagnosed with SLD, from a free-range laying chicken farm in Seymour, Victoria, Australia. Dead chickens were necropsied by a registered veterinarian for routine diagnostic purposes. All other methods were performed in accordance with the standard guidelines and regulations for a physical containment level 2 (PC2) laboratory. The Animal Ethics Committee at La Trobe University was informed that findings from the diagnostic material were to be used in a publication, and a formal waiver of ethics approval was granted. Viral nucleic acids were extracted using a QIAamp viral RNA minikit (Qiagen, USA) without carrier RNA, which allowed the simultaneous extraction of viral DNA and RNA. The library was prepared using an Illumina DNA preparation kit, starting with 250 ng of DNA (6). The quality and quantity of the prepared library were assessed by the Australian Genome Research Facility (AGRF) (Melbourne, Australia), and the library was sequenced with the Illumina NovaSeq sequencing platform, generating 150-bp paired-end reads.

Sequencing data were analyzed with an established pipeline (13–16) using Geneious Prime (version 2022.1.1; Biomatters, New Zealand) and CLC Genomics Workbench (version 9.0.1). Briefly, 47.73 million raw reads were preprocessed to remove the Illumina adapters, ambiguous base calls, and poor-quality reads (trim using quality score limit of 0.05 and trim ambiguous nucleotides up to 15 using CLC Genomics Workbench), followed by mapping against the genomes of chickens (Gallus gallus) (GenBank accession number NC_006088.5) and Escherichia coli (GenBank accession number U00096) to remove nonviral DNA. A total of 45.5 million cleaned and unmapped reads were used as input data for the de novo default assembler in CLC Genomics Workbench (version 9.0.1). This resulted in the generation of a linear 4,367-bp contig, identified as a GaChPV-4 genome (based on similarity to the reference sequence...
of GaChPV-3 [GenBank accession number MW306779.1], with average coverage of 178.1 ×. Annotation of the assembled genome was performed using Geneious Prime (version 2022.1.1). All software was used with default parameters except where stated.

The genome is 4,367 bp long, with 21-nucleotide inverted terminal repeat sequences and with a G+C content of 39.9%. The GaChPV-4 contained four open reading frames (ORFs) (Fig. 1A), and comparative analysis of the predicted ORFs was conducted by using BLASTx and BLASTp with GenBank (17). The ORFs encoding NS1 and VP1 proteins share 77.63% and 70.02% amino acid identity, respectively, with the corresponding proteins of GaChPV-3 (GenBank accession number MW306779.1). Phylogenetically, GaChPV-4 shows obvious evolutionary relationships with other GaChPVs (Fig. 1B).

Like other paroviruses, the complete NS1 gene of GaChPV-4 is 674 amino acids long and encodes a helicase, including the conserved ATP- or GTP-binding Walker A loop (GPxNTGKT/S[322]GPxNTGKS[329]), Walker B (xxxWEE[360]WGKWEEx[363]), Walker B’ (KQxxEGxxxxxPxK[377]QKIEGMETHTIPxK[380]), and Walker C (PoxxTxN[400]PIWITTTxN[403]) amino acid motifs. In addition, the NS1 protein contains two conserved replication initiator (endonuclease) motifs, i.e., xxHuHxxxx (IF112HIV115GLCK) and YxxK (169YMC172) (conserved amino acids are indicated in bold letters, and u indicates a hydrophobic residue).

This study reports evidence of a novel GaChPV-4 in a free-range laying chicken that was clinically diagnosed with SLD. Additional investigations will be required to better understand the host-pathogen dynamics, including routes of transmission, associated pathology, and disease prevalence.
**Data availability.** The complete GaChPV-4 genome sequence from the free-range laying chicken has been deposited in DDBJ/ENA/GenBank under the accession number OM920501. The version described in this paper is the first version, OM920501.1. The raw sequencing data from this study have been deposited in the NCBI Sequence Read Archive (SRA) under the accession number SRR19134919 (BioProject accession number: PRJNA835504).

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**REFERENCES**

1. Pénzes JJ, Soderlund-Venermo M, Canuti M, Eis-Hübinger AM, Hughes J, Cotmore SF, Harrach B. 2020. Reorganizing the family Paroviridae: a revised taxonomy independent of the canonical approach based on host association. Arch Virol 165:2133–2146. https://doi.org/10.1007/s00705-020-04632-4.

2. Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, Qiu J, Soderlund-Venermo M, Tattersall P, Tijsen P, Gatherer D, Davison AJ. 2014. The family Paroviridae. Arch Virol 159:1239–1247. https://doi.org/10.1007/s00705-013-1914-1.

3. Phan TG, Guillan F, Simeone C, Deng X, Delwart E. 2015. Sesavirus: prototype of a new parvovirus genus in feces of a sea lion. Virus Genes 50:134–136. https://doi.org/10.1007/s11262-014-1123-3.

4. Duarte MA, Silva JMF, Brito CR, Teixeira DS, Melo FL, Ribeiro BM, Nagata T, Campos FS. 2019. Faecal virome analysis of wild animals from Brazil. Viruses 11:803. https://doi.org/10.3390/v11090803.

5. Hargitai R, Boros Á, Pankovics P, Mátics R, Altan E, Delwart E, Reuter G. 2021. Detection and genetic characterization of a novel parvovirus (family Paroviridae) in barn owls (Tyto alba) in Hungary. Arch Virol 166:231–236. https://doi.org/10.1007/s00705-020-04862-6.

6. Sarker S. 2021. Metagenomic detection and characterisation of multiple viruses in apparently healthy Australian Neophema birds. Sci Rep 11:20915. https://doi.org/10.1038/s41598-021-00440-1.

7. Wille M, Shi M, Hurt AC, Klaassen M, Holmes EC. 2021. RNA virome abundance and diversity is associated with host age in a bird species. Virology 561:98–106. https://doi.org/10.1016/j.virology.2021.06.007.

8. Sarker S. 2021. Molecular and phylogenetic characterisation of a highly divergent novel parvovirus (psittaciform chaphamaparvovirus 2) in Australian Neophema parrots. Pathogens 10:1559. https://doi.org/10.3390/pathogens10121559.

9. Palinski RM, Mitra N, Hause BM. 2016. Discovery of a novel Parovovirusine virus, porcine paroviruses 7, by metagenomic sequencing of porcine rectal swabs. Virus Genes 52:564–567. https://doi.org/10.1007/s11262-016-1322-1.

10. Roediger B, Lee Q, Tikoo S, Cobbin JCA, Henderson JM, Jormakka M, O’Rourke MB, Padula MP, Pinello N, Henny M, Wymne M, Santagostino SF, Brayton CF, Rasmussen L, Lisowski L, Tay SS, Harris DC, Bertram JF, Dowling JP, Bertolino P, Lai JH, Wu W, Bachovchin WW, Wong JJ, Gorrell MD, Shaban B, Holmes EC, Jolly CJ, Monette S, Weninger W. 2018. An atypical parvovirus drives chronic tubulointerstitial nephropathy and kidney fibrosis. Cell 175:530–543.e24. https://doi.org/10.1016/j.cell.2018.08.013.

11. Matos M, Blic I, Viloux N, Albaric O, Chatenet X, Tvarogová J, Dinhopf N, Liehdl S, Hess M. 2022. A novel Chaphamaparvovirus is the etiological agent of hepatitis outbreaks in pheasants (Phasianus colchicus) characterized by high mortality. Transbound Emerg Dis 69:e2093–e2104. https://doi.org/10.1111/tbed.14545.

12. Sarker S, Athukorala A, Phalen DN. 2022. Characterization of a near-complete genome sequence of a chaphamaparvovirus from an Australian boobook owl (Ninox boobook). Microb Resour Announc 11:e00249-22. https://doi.org/10.1128/mra.00249-22.

13. Sarker S, Das S, Lavers JL, Hutton I, Helbig K, Imbery J, Upton C, Raidal SR. 2017. Genomic characterization of two novel pathogenic avipoxviruses isolated from Pacific shearwaters (Ardenna spp.). BMC Genomics 18:298. https://doi.org/10.1186/s12864-017-3680-z.

14. Athukorala A, Phalen DN, Das A, Helbig KJ, Forwood JK, Sarker S. 2021. Genomic characterisation of a highly divergent siadenovirus (psittacine siadenovirus F) from the critically endangered orange-bellied parrot (Neophaea chrysogaster). Viruses 13:1714. https://doi.org/10.3390/v13091714.

15. Sutherland M, Sarker S, Vaz PK, Legione AR, Devlin JM, Macwhirter PL, Whiteley PL, Raidal SR. 2019. Disease surveillance in wild Victorian cacatuids reveals co-infection with multiple agents and detection of novel avian viruses. Vet Microbiol 235:257–264. https://doi.org/10.1016/j.vetmic.2019.07.012.

16. Sarker S, Isberg RS, Moran LJ, Araujo DR, Elliott N, Melville L, Beddoe T, Helbig JK. 2019. Crocidolopex virus evolutionary genomics supports observed poxvirus infection dynamics on saltwater crocodile (Crocodylus porosus). Viruses 11:1116. https://doi.org/10.3390/v11121116.

17. Benson DA, Cavanaugh M, Driskell MT, Gradoville J, Teeling E, Statham P, Campbell F, Lai JH, Chang Y, Raymond D, Tang X, Ahmadzadeh F, Blanchette-Mottley Y, Dinh P, Tang Q, Haavikko M, Wang Z, Alcosta-Králik P, Carrington R, Cuff J, Furey T, Grant S, Han C, Heger A, Helm K, Ishikawa Y, Kasif S, Keane TM, Keightley SD, Kim Y, Minnich L, Moult J, Nekrutenko A, O’Connor T, O’Shea M, Osorio R, Pagni D, Pachter L, Parra M, Paweletz A, Reid J, Rice A, Sallabio E, Shumway M, Skarlatos S, Solomon I, Starnes S, Stoye J, Szilagyi M, Tsai J, Vaninetti J, Vira R, West M, White S, Willms C, Yee LM, Zhang D, Zhang Y, Zhao H, Zirbel C, Zody MC, Bork P. 2019. The complete genome sequence from the free-range laying chicken has been deposited in DDBJ/ENA/GenBank under the accession number OM920501. The version described in this paper is the first version, OM920501.1. The raw sequencing data from this study have been deposited in the NCBI Sequence Read Archive (SRA) under the accession number SRR19134919 (BioProject accession number: PRJNA835504).