Complete Genome Sequence of Infectious Bronchitis Virus Strain JP/KH/64, Isolated in Japan

Masaji Mase, Kanae Hiramatsu, Satoko Watanabe, Hiroshi Iseki

National Institute of Animal Health, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

Oita Livestock Hygiene Service Center of Oita Prefecture, Oita, Oita, Japan

United Graduate School of Veterinary Sciences, Gifu University, Gifu, Gifu, Japan

ABSTRACT Here, we report the complete genome sequence of infectious bronchitis virus (IBV) strain JP/KH/64, which is the reference strain for the JP-I genotype in Japan. This information should be useful for an in-depth understanding of the evolution of the JP-I genotype.

A vian infectious bronchitis virus (IBV) belongs to the genus Gammacoronavirus in the family Coronaviridae of the order Nidovirales. This highly contagious pathogen is transmitted through the respiratory tract and causes infectious bronchitis in chickens, leading to serious economic consequences worldwide. IBV has three major virus-encoded structural proteins, i.e., the spike (S) glycoprotein, the membrane (M) protein, and the envelope (E) protein.

### Table 1: Primers used to amplify the complete genome sequence of JP/KH/64

| Nucleotide positions | Forward primer | Reverse primer |
|----------------------|----------------|----------------|
| 19–1645              | TATATATCTATTGCACCTAGCC | AGTCAGACAGACAACAGCT |
| 1437–3080            | CACAAGTGTTTGCAGGCTGAG | GAGGCTCCTTTATTGAACATAC |
| 2893–3851            | AGATGCTGGAGATTGCAGTAC | GGTTACTGTCTCCCACTCCGG |
| 3742–5610            | GGATGCTGGTAACCTAGGCGC | GTACCACTAACGATACCC |
| 5438–7344            | TGTTGTACTGCAGCAGCAC | TACACCCTAAGAGCAAGC |
| 7223–9001            | TCAGGACTGTCGAAGTCAGC | TACCACCATAGAGCAAGC |
| 9176–10950           | GAAAGCAAATCAGTCATGATAG | GAGGGATGTGGAAACACT |
| 10816–12586          | GGCAAAATTGGTGATGGAAG | ATTAGTGGGAGCAACAG |
| 12412–14028          | CCTGATGTTGATAGAGCTCC | AAGAATGGGAAACAGCAG |
| 13150–14095          | FCCCTTCGAACTATATGATT | ATAGAAGAGAGGCACAG |
| 13929–14875          | TACACAGGAAAGTTCTGAGC | CATCTCAGAAACACATGC |
| 14813–15647          | AGCCTAAATCCTTCGCACTCC | TTACCGGCTCCTCTGAGATAG |
| 15520–16688          | CTATTACAGCCTTTGCTCTG | ATGACTTACATAGTACCC |
| 16738–19004          | GTAGACCTTCTACAAAGTTG | TAAACATACAGATCCGTC |
| 17318–18797          | TAGGCAAAATTTTGGAGCCTG | TTAACAGATTACCATATAAGG |
| 18402–20306          | GGCTTCTTATAATGCACTG | AAATTCACACAGTGGTTTC |
| 20071–22297          | CAAGATTGTGCACTGTCAGG | TATGTTATCACAACAGGAC |
| 20959–21902          | CTTAAAAGCAGGCGACATCCTACAAT | TACACACCTGTAATACAAATGCT |
| 21275–22976          | ACACAAACACGCTCAGAGTGG | CTGAAATATGCAACTGATAG |
| 22801–24896          | CATGATGTTGATACTGAGGGAAG | GGAGTATTGAAACCTACGAC |
| 23757–24991          | GTGGTGGTTGCAATCTGCTG | CTGGACCTTCAAATAAGAAGAC |
| 24764–25867          | GCAGGAAATCCATATCGT | TCTGCTTCTCCATCCTGG |
| 25089–26426          | GTCAGCGAAGCGAATAATAA | TCAAGGAGAATGAGCTCCAC |
| 26264–27672          | GTGAGTATTCTAAAGATTTGATAT | GCCTAACCTCTATATAGCT |
| 1–157                | AUAPA ‡ | AAAACCGTGACAGGTTGCCAG |
| 1–202                | AAP‡ | GGCGAAAAACAGACATGATA |
| 27448–27672          | TTATGAGCTGGAACACCTA | UAPA ‡ |

aPrimer locations are listed according to the JP/KH/64 strain.
bThe abridged universal amplification primer (AUAP), 5′-RACE abridged anchor primer (AAP), and universal amplification primer (UAP) are included in the commercial RACE kit (Invitrogen).

Citation Mase M, Hiramatsu K, Watanabe S, Iseki H. 2021. Complete genome sequence of infectious bronchitis virus strain JP/KH/64, isolated in Japan. Microbiol Resour Announc 10:e00665-21. https://doi.org/10.1128/MRA.00665-21.

Editor John J. Dennehy, Queens College CUNY

Copyright © 2021 Mase et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Masaji Mase, masema@affrc.go.jp.

Received 1 July 2021
Accepted 1 September 2021
Published 7 October 2021
Among these proteins, the S1 glycoprotein is associated with virus attachment and is a major target of neutralizing antibodies in chickens (2–4). In Japan, seven genotypes of IBV (JP-I, JP-II, JP-III, JP-IV, Mass, Gray, and 4/91) have been confirmed, based on the partial nucleotide sequence of the S1 gene (5, 6). Analysis of genes other than S1 revealed that various recombinant viruses are also prevalent in Japan (6). A complete genome analysis of the IBV is important for understanding the epidemiology of recombinant viruses. To understand the epidemiology of variant IBV in Japan, the complete nucleotide sequence of the major genotype JP-I strain was determined.

Figure 1: Phylogenetic tree based on the complete S1 glycoprotein gene of IBV. Nucleotides 20368 to 21978 (1,632 bases) of the S1 gene of IBV Beaudette (GI-1) (GenBank accession number NC_001451) were subjected to phylogenetic analysis. The phylogenetic tree was generated using the neighbor-joining method in MEGA 7 (14) with 1,000 bootstrap replications. All tools were run with default parameters unless otherwise specified. Horizontal distances are proportional to the minimum number of nucleotide differences required to join nodes and sequences. The genotypes of IBV were defined by Valastro et al. (10). The JP/KH/64 strain is indicated with a black circle.
The JP/KH/64 strain was isolated from chickens with respiratory symptoms in 1964 in Japan and was first confirmed as the JP-I genotype in Japan (5). The virus was grown in primary chicken kidney cell cultures prepared from 4- to 10-week-old chicks, and the infected culture fluids were harvested. Viral RNA was extracted from infected culture fluids using a QIAamp viral RNA minikit (Qiagen, Hilden, Germany), and random hexamer primers were used for cDNA synthesis (6). Specific primers for genome sequencing using the Sanger method were designed based on previous studies (7, 8) and on the sequences obtained from amplicons (Table 1). Both the 3’ and 5’ termini of the genome were determined using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique. The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.

The sequenced fragments were assembled using ATGC-Mac v.5 (Software Development, Japan). All tools were run with default parameters unless otherwise specified. The length of the complete genome of JP/KH/64 was 27,672 nucleotides (nt), with a G+C content of 38.20%, excluding the poly(A) tail. The open reading frames (ORFs) were identified using the rapid amplification of cDNA ends (RACE) technique.