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NOTE

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Identification of domestic cat hepadnavirus from a cat blood sample in Japan

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ABSTRACT. The hepatitis B virus (*Hepadnaviridae*) induces chronic hepatitis and hepatic cancer in humans. A novel domestic cat hepadnavirus (DCH) was recently identified in several countries, however, the DCH infection status of cats in Japan is unknown. Therefore, we investigated the DCH infection rate of 139 cat samples collected in Japan. We identified one positive blood sample (0.78%) from a 17-year-old female cat with chronically elevated alanine aminotransferase. Phylogenetic analysis demonstrated that the DCH strain identified in this study is genetically different from strains in other countries. Further investigations are required to elucidate the evolution of DCH and the impact of DCH infection on hepatic diseases in domestic cats.

KEYWORDS: domestic cat hepadnavirus (DCH), hepatic virus, pet cat

Hepatitis B virus (HBV) (*Hepadnaviridae*) is one of the causative agents of chronic hepatitis and hepatocellular carcinoma. Despite effective vaccines against HBV, over 350 million people worldwide are chronically infected with HBV [4]. One step in the life cycle of HBV involves the conversion of viral pregenomic RNA to a relaxed circular DNA; therefore, several reverse transcriptase inhibitors in combination with interferons are currently used for controlling HBV infection [9].

It has been unclear whether other mammals can be infected by similar hepadnaviruses that induce chronic hepatitis and hepatocellular carcinoma. In 2018, an Australian group identified a novel feline hepadnavirus, the domestic cat hepadnavirus (DCH), and since then, it has been detected in cats in several countries including Australia (6.5% of all tested samples) [1] and Italy (10.8% of all tested samples) [8]. Although it remains unclear whether DCH can induce hepatic diseases in cats, the detection of DCH has been associated with chronic hepatitis and hepatocellular carcinoma [10]. In addition, cats infected with the feline immunodeficiency virus (FIV) also tend to be positive for DCH [1], suggesting that immunodeficiency induced by FIV may lead to infection with DCH.

The fact that DCH has been discovered in several countries suggests that it is prevalent worldwide. Phylogenetic analyses in previous studies suggest that DCH is divergent among strains from different countries, suggesting that DCH has been evolving in each country. So far, DCH has been identified Australia [1], Thailand [11], Italy [10], the United Kingdom [7], and Malaysia [2]. Its presence and prevalence in Japan, however, remains undetermined. In this study, therefore, we investigated the presence and prevalence of DCH in Japan.

We screened 139 blood samples collected from several clinics in Japan. The blood samples were collected from cats that were mainly housed indoors. We used samples that were left over after performing routine blood tests. Prior to testing for DCH,

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we obtained the owner's informed consent. This study was approved by the Animal Experiment Committee of the University of Miyazaki (authorization number: 2021-019). All experiments were performed in accordance with relevant guidelines and regulations.

All collected blood samples were mixed with the anticoagulants, heparin or ethylenedinitrilo-tetraacetic acid disodium (EDTA/2Na). In this study, the blood samples were directly subjected to PCR without extracting DNA. Previous studies have demonstrated that heparin inhibits PCR [6], thus we used a KOD One PCR Master Mix (TOYOBO, Osaka, Japan), which is relatively tolerant to heparin contamination. To confirm that the PCR reaction was not blocked by anticoagulants, we used primer pairs to amplify the cat glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) housekeeping gene. The primers used to amplify the DCH genome are DCH-F: (5′-ATTCAAGCGCTCTATGAAGAGG-3′) and DCH-R: (5′-AAAAGTGAGGCAAGAGAGATGG-3′), while those used to amplify cat *Gapdh* are GAPDH-F: (5′-CCTTCATTGACCTCAACTACAT-3′) and GAPDH-R: (5′-CCCCAGTAGACTCCACAACATAC-3′). The PCR conditions for both genes were 40 cycles of 98°C for 10 sec, 60°C for 5 sec, and 68°C for 10 sec, followed by 68°C for 7 min. We used a synthesized DNA fragment encoding the partial DCH genome (Eurofins, Tokyo, Japan) as a positive control for DCH. The amplicons were visualized on a 1.5% agarose gel.

We detected *Gapdh* amplicons from the 128 samples tested. A single sample (#116) (0.78% of tested samples) was positive for DCH (Fig. 1). We then sequenced the entire viral genome using six pairs of primers whose sequences are listed in Table 1. We designed the primers on the Primer3 website (https://bioinfo.ut.ee/primer3-0.4.0/) [accessed on Jan. 8, 2022]. All six fragments were efficiently amplified. Each amplicon was run on an agarose gel and then extracted from the gel using the QIAquick Gel Extraction Kit (Qiagen, Tokyo, Japan). The sequences of the amplicons were determined using a BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Tokyo, Japan) on an Applied Biosystems 3130xl DNA Analyzer (Thermo Fisher Scientific). The sequence assembly was performed using 4Peaks (Nucleobytes, Aalsmeer, The Netherlands) and Microsoft Word 2019 (Microsoft, Redmond, WA, USA). The assembled sequence was deposited in GenBank (Accession# LC668427).

The sequence of our strain was aligned with those of 15 other DCH strains and the sequences were analyzed phylogenetically using MEGA X (MEGA Software). We used the protein sequences of hepatitis B virus serotype adr (AY123041.1) as an outer group. Our phylogenetic analysis revealed that the amino acid sequences of the polymerase, surface, and core proteins of Domestic cat hepadnavirus Japan/KT116/2021 identified in this study were genetically close to those of previously reported strains (Fig. 2). In contrast, the amino acid sequence of the X protein of Domestic cat hepadnavirus Japan/KT116/2021 was different from

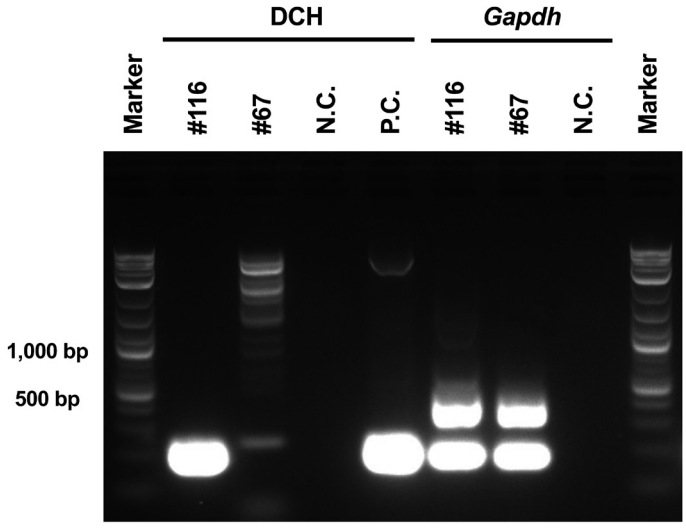

Fig. 1. PCR screening of blood samples from cats to detect the domestic cat hepadnavirus (DCH) genome. Heparinized blood was used to amplify DCH and the cat *Gapdh* gene. Marker, N.C., and P.C. denote DNA size marker, negative control, and positive control, respectively.

Table 1. Primers used for determining viral whole genome

Fragment	Forward primer	Reverse primer
#1	5′-ACTCTCAAACAGGGAACATTCGT-3′	5′-CATCCGACCGGAATAATAATTAAC-3′
#2	5′-AATTCTCCAAAGGCTAACAGGTTTA-3′	5′-ATTCCACCAATAGCAGATCACGTAG-3′
#3	5′-TACGTCCCTTCCACTCTGAATC-3′	5′-CAAGACAGTATGTTGTCCAAAAGTG-3′
#4	5′-GAAGAGGAACTTACAGGTAGGGAAC-3′	5′-GTCTAGATTGTGACGAGGGAAAAAC-3′
#5	5′-CTCGATACCCTGATTATTCTCTTCA-3′	5′-CCCTATTGTTTGTATTTTTGTCCAC-3′
#6	5′-CAGTTGGAGACAGAAGTACGGTTAT-3′	5′-CATCCATATAAGCAAACACCATACA-3′

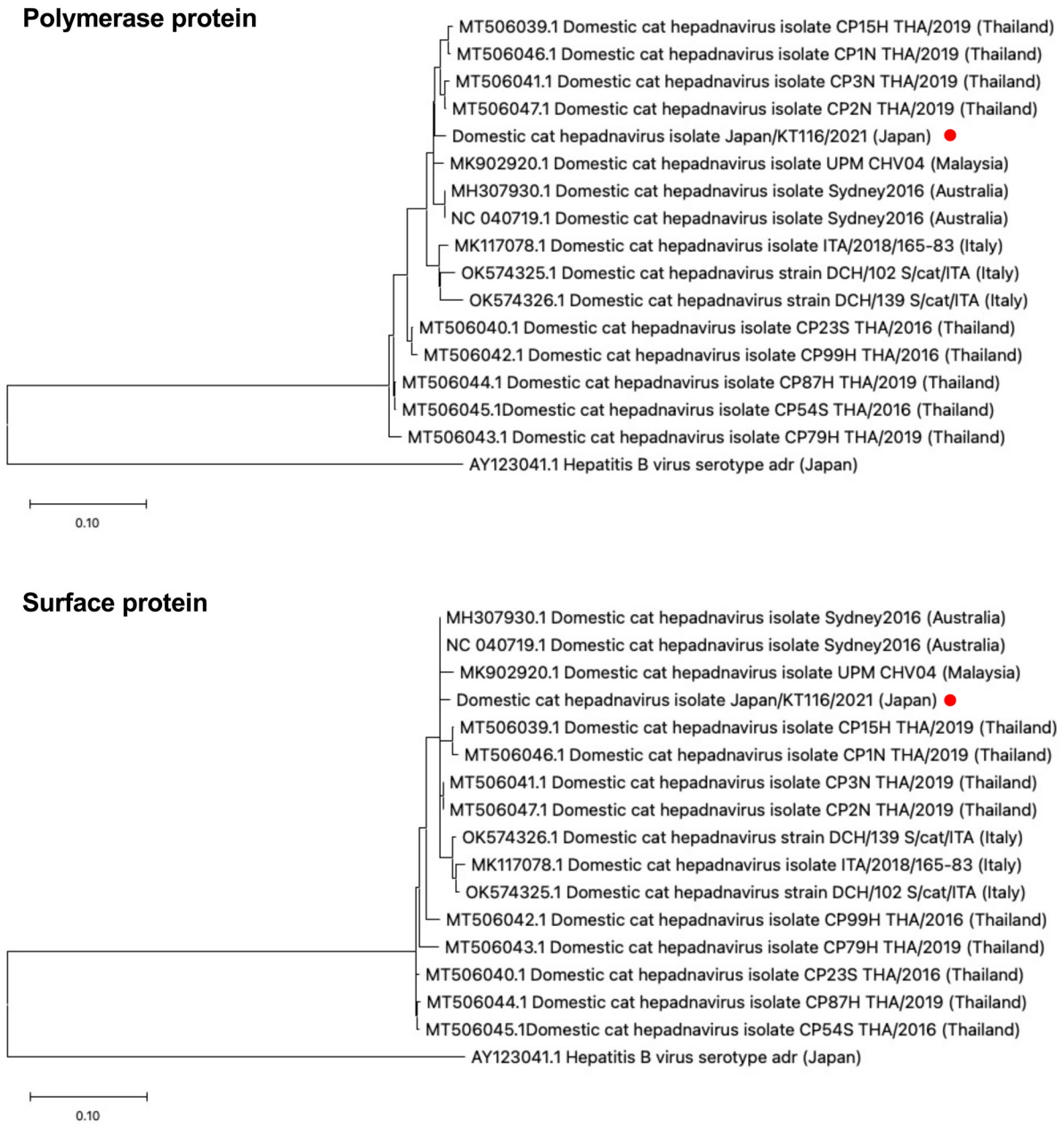

Fig. 2. Phylogenetic analysis of domestic cat hepadnavirus (DCH)/Japan/KT116/2021. The phylogenetic position of Domestic cat hepadnavirus (DCH)/Japan/KT116/2021 within the family *Hepadnaviridae*. The maximum likelihood tree is based on each hepadnaviral protein sequences retrieved from the GenBank database. The DCH/KT116/2021 (Accession number LC668427) is indicated by a red dot. The tree is drawn to scale, thus the branch lengths correspond to the number of substitutions per site.

those of other strains. We used Protein BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) [accessed on Feb. 14, 2022] to calculate the sequence identity of X protein between Domestic cat hepadnavirus Japan/KT116/2021 and other strains. Its identity with Domestic cat hepadnavirus isolate Sydney2016 (MH307930.1, identified in Australia) was 95.9%, whereas that with Domestic cat hepadnavirus isolate CP3N_THA/2019 (MT506041.1, identified in Thailand) was 93.1%. The X protein of HBV (HBx) plays roles in silencing host antiviral defenses and promoting viral transcription [3, 12]. Therefore, it will be important to elucidate the impact of polymorphism in the DCH X protein.

The source of the DCH-positive sample (#116) was a 17-year-old female cat (Cat #116) born in Japan and with no record of traveling overseas. Therefore, Cat #116 was likely infected with DCH in Japan. At the time of the PCR test, Cat #116 had no health problems, based on interviews and physical examinations. However, prior to its death due to acute neuropathy, we observed a persistent elevation of alanine aminotransferase (ALT) (Fig. 3). Moreover, Cat #116 underwent a splenectomy one year before the PCR test, and was then diagnosed with a mast cell tumor. After this diagnosis, the cat was treated with CCNU, an anticancer

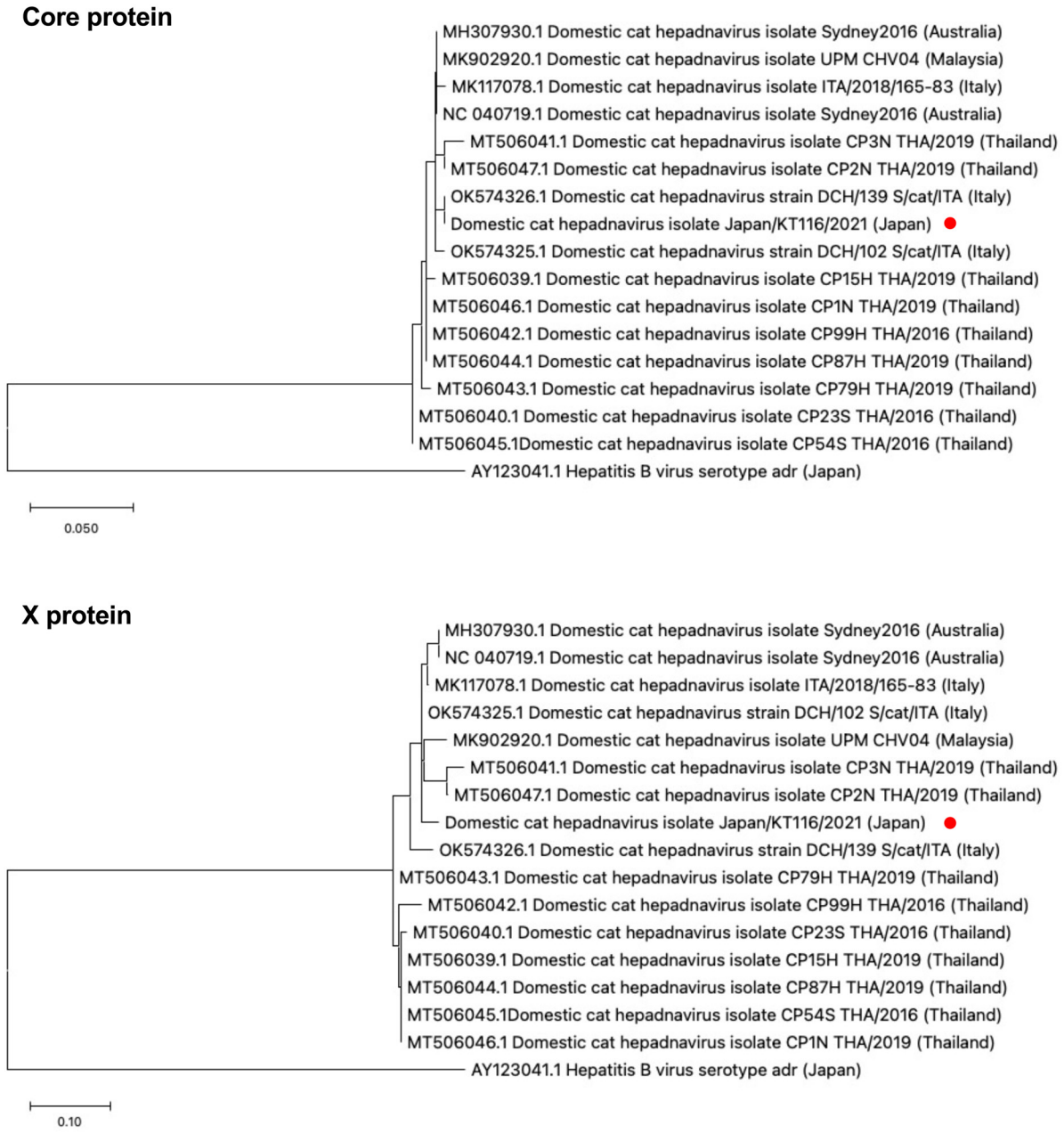

Fig. 2. Continued.

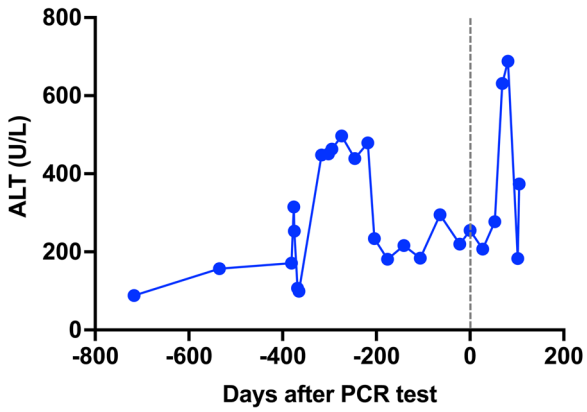

Fig. 3. Changes in alanine aminotransferase (ALT) levels in Cat #116. A plot of ALT levels in each blood test. The X-axis represents the number of days after PCR testing.

drug. While we cannot exclude the possibility that the treatments against the mast cell tumor induced elevated levels of ALT, it is also possible that DCH infection had affected the health status of Cat #116. Currently, it is not known whether the mast cell tumor itself or the treatments against mast cell tumor had induced immunosuppression in Cat #116, leading to infection with DCH. As Cat #116 was tested positive for DCH after its death, we were unable to perform an autopsy. Cat #116 was negative for FIV and feline leukemia virus.

In conclusion, to the best of our knowledge, this is the first time that DCH has been detected in a cat in Japan. The prevalence of DCH in this study (0.78%) is lower than those of previous studies in other countries [1, 8]. Further investigation is required to determine the reason for this difference. As most of samples in this study were collected from indoor cats, future studies must aim to test the prevalence of DCH among outdoor cats. The results of phylogenetic analysis of the X protein reveal that Domestic cat hepadnavirus Japan/KT116/2021 is genetically different from previously described strains from other countries, suggesting that Domestic cat hepadnavirus Japan/KT116/2021 strain is native to Japan. Because homologous recombination has been reported with HBV [5, 13] and DCH [11], it is important to monitor the infection status and evolutionary history of DCH in every country with a large domestic cat population. Also, the impact of DCH infection on chronic hepatitis in cats should be elucidated.

CONFLICTS OF INTEREST. The authors declare no conflict of interest.

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