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Chronic Domestic Cat Hepadnavirus Infection in a Feline Patient with Liver Disease: Integrated Serological, Molecular, and Genomic Diagnosis

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ABSTRACT

A stray female domestic shorthair cat, approximately three years of age, presenting with biochemical and clinical evidence of liver dysfunction, was confirmed to be infected with domestic cat hepadnavirus (DCH). Integration of molecular testing and antibody profiling suggested a chronic infection phase, as IgM anti-core antibodies—commonly linked to acute Hepatitis B Virus (HBV) cases—were undetectable. In contrast, IgG anti-core antibodies were identified, consistent with chronic HBV infections in humans. The absence of anti-DCH surface antibodies indicated no seroconversion or long-term immune protection. Genomic analysis demonstrated 98.3% sequence similarity to Italian DCH isolates.

Keywords: Feline, DCH, Serum profile, Hepatic disease, Hepatitis

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Introduction

Domestic cat hepadnavirus (DCH), first identified in 2018, shows close evolutionary kinship to human Hepatitis B Virus (HBV). Its genome, roughly 3.2 kilobases long, consists of circular, partially double-stranded DNA [1]. Research across Asia, Australia, the United States, and Europe reports DCH detection in 0.2–18% of cat samples [2–7]. Still, these rates likely underrepresent their true prevalence, as anti-DCH core antibodies (anti-DCHc) have appeared in nearly one-quarter of tested felines [8]. The biological behavior and disease mechanisms of DCH remain unclear, though it has been proposed as a factor in feline hepatic disorders. Pathological lesions compatible with chronic inflammation and tumor formation—similar to HBV-induced changes—have been documented in DCH-positive cats diagnosed with persistent hepatitis or hepatocellular carcinoma (HCC) [9]. Moreover, an association between hepatic enzyme abnormalities and DCH DNA presence has been reported [2–4, 6, 10–17]. DCH DNA has also been found in serum and both inflammatory and non-inflammatory peritoneal fluids, even when absent in liver biopsies from infected cats showing hematologic and biochemical irregularities [5]. Some infected cats exhibit viremia with either normal or slightly raised liver enzyme activity [18]. Age over two years and concurrent feline retroviral infection are recognized as additional risk elements for DCH circulation [3, 6, 8, 10, 17]. HBV infection in humans shows variable progression—from mild acute cases to long-term chronic hepatitis [19]. Diagnostic evaluation typically includes antigen and antibody detection, along with viral DNA quantification and blood parameter analysis [19, 20]. A similar diagnostic model may be advantageous for DCH.

This report presents a clinical case of DCH infection in a cat with liver disease, confirmed by both molecular and serologic methods, and includes complete viral genome sequencing.

Case description

In March 2021, a three-year-old female stray domestic short-haired (DSH) cat was brought to a veterinary clinic for lethargy, inappetence, and weakness. The physical exam showed a normal body temperature (38 °C), pale mucosa, jaundice, and weight reduction. A diagnostic baseline was established through complete blood count, biochemical profile, urinalysis, and retrovirus screening using a point-of-care immunochromatographic assay (SNAP FIV/FeLV Combo Test, IDEXX). Serum chemistry revealed elevated alkaline phosphatase (ALP; 160 IU/L; reference 14–62), gamma-glutamyl transferase (GGT; 6.2 IU/L; reference 0.0–1.0), total bilirubin (4.42 mg/dL; reference 0.14–0.25), globulins (5.5 g/dL; reference 2.9–4.3), γ-globulin (33.6%; reference 15.0–28.0), and amylase (1755 IU/L; reference 648–1262). Meanwhile, cholesterol (91 mg/dL; reference 112–194), total iron (24 µg/dL; reference 55–152), and saturation (6.8%; reference 20–53) were decreased. The hematologic analysis indicated leukocytosis (WBC 46.7 × 10^3/µL; normal 5.5–12 × 10^3/µL) and anemia (RBC 3.51 × 10^6/µL; normal 6.0–9.5 × 10^6/µL). Retrovirus testing was negative. Over four follow-up evaluations (April, October, November 2021, and February 2022), the animal persistently exhibited elevated ALP, GGT, bilirubin, and WBC levels, along with ongoing anemia and leukocytosis. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values exceeded reference limits at two and three points, respectively **(Table 1)**.

Table 1. Hematologic and biochemical data of the patient. Abnormal values are marked in bold.

Sampling Date	Red Blood Cells (RBC) 6.0–9.5 × 10^6/µL	White Blood Cells (WBC) 5.5–12 × 10^3/µL	Platelets (PLT) 130–400 × 10^3/µL	Creatine Phosphokinase (CPK) 52–295 IU/L	Aspartate Aminotransferase (AST) 16–46 IU/L	Alanine Aminotransferase (ALT) 33–70 IU/L	Alkaline Phosphatase (ALP) 14–62 IU/L	Gamma-Glutamyl Transferase (GGT) 0–1 IU/L	Total Bilirubin 0.14–0.25 mg/dL	DCH DNA	Notes
18 March 2021	3.51	46.7	382	146	26	30	160	6.2	4.42	NT	
9 April 2021	5.11	60.5	397	62	30	101	94	5.0	4.16	NT	
14 October 2021	6.33	27.7	482	68	167	162	63	2.8	1.87	NT	
25 November 2021	5.78	23.8	256	76	20	38	76	4.0	1.49	Pos.	Liver biopsy positive for hepadnavirus (2.9 × 10^1 DNA copies/mL)
11 February 2022	3.68	25.7	348	148	183	1285	93	4.1	0.31	Neg.	

Abbreviations: RBC—red blood cells; WBC—white blood cells; PLT—platelets; CPK—creatine phosphokinase; AST—aspartate transaminase; ALT—alanine transaminase; ALP—alkaline phosphatase; GGT—gamma-glutamyl transferase; IU—International Unit; L—Liter; mg—milligram; µL—microliter; mL—milliliter; DCH—domestic cat hepadnavirus; Neg.—negative; Pos.—positive; NT—not tested.

Abdominal ultrasound scans performed in March, April, and November 2021 **(Figure 1)** revealed findings consistent with chronic hepatopathy and gallbladder irregularities with mild bile stasis.

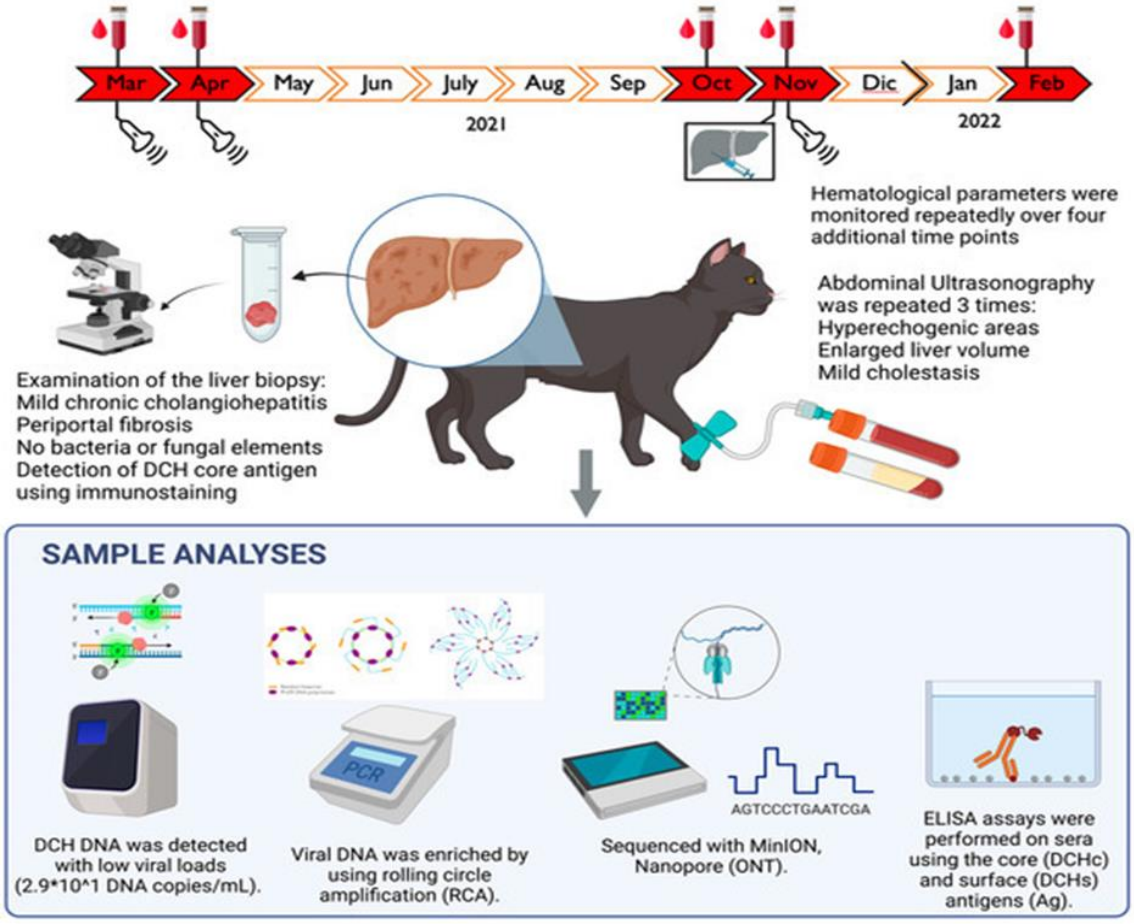
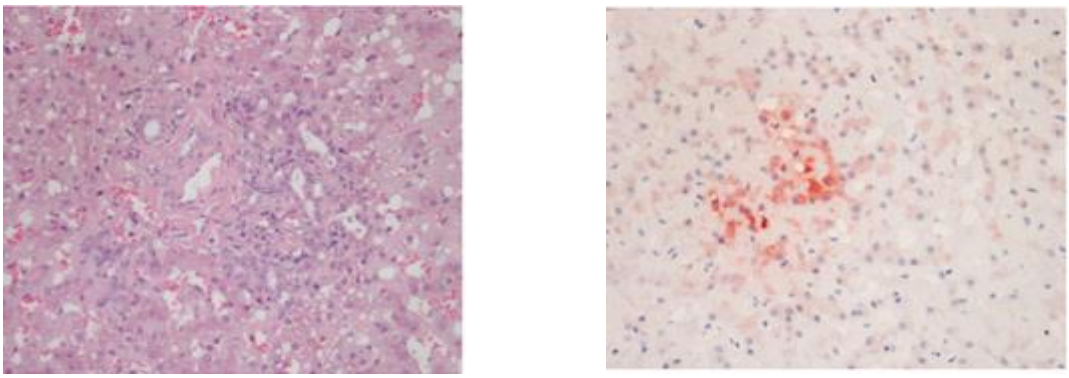


Figure 1. Diagnostic workflow stages.

A liver biopsy sample obtained in November 2021 was preserved in 10% neutral buffered formalin, processed into paraffin blocks, and stained with hematoxylin and eosin. Microscopically, the tissue exhibited moderate fibrosis in nearly all portal tracts, accompanied by mild lymphoplasmacytic infiltration and ductular hyperplasia—features indicative of mild chronic periportal hepatitis **(Figure 2a)**. No microbial organisms were detected. Quantitative PCR (qPCR) testing [10] performed on the biopsy and two serum samples (collected in November 2021 and February 2022) detected DCH DNA solely in the liver specimen, with an estimated viral load of 2.9×10^1 DNA copies/mL.



a) b)

Figure 2. (a) Mild chronic periportal hepatitis with ductular proliferation (H&E, 400×); (b) focal cytoplasmic immunolabeling using DCH immunohistochemistry (400×).

When all three samples were retested using a qualitative PCR method [1], both the liver tissue and the serum obtained in November 2021 yielded positive results, while the February 2022 serum sample—collected roughly

three months after the first diagnosis—was negative for DCH. None of the analyzed materials contained nucleic acids from feline calicivirus, herpesvirus type 1, parvovirus (panleukopenia), coronavirus, or retroviruses, excluding the possibility of concurrent infections [21–26]. Immunohistochemical (IHC) evaluation of the liver biopsy, conducted with a rabbit polyclonal antibody targeting the HBV core antigen (HBcAg) (Thermo Fisher Scientific, Waltham, MA, USA) following previous methodologies [11, 12], demonstrated localized strong cytoplasmic reactivity **(Figure 2b)**, supporting the presence of DCH core antigen (DCHcAg).

To further characterize the DCH isolate, viral DNA from the liver sample was amplified through rolling circle amplification (RCA) using a TempliPhi kit with RCA primers. A 1 µg aliquot (1:100 dilution) of the purified RCA product was subsequently used as input for library construction with the Ligation Sequencing Kit 1D SQK-LSK110 (ONT™, Oxford, UK) and sequenced using the MinION platform (Oxford Nanopore Technologies™). Genome assembly was performed in Geneious Prime v2021.2 (Biomatters Ltd., Auckland, New Zealand). The DCH genome was aligned with sequences retrieved from GenBank (NCBI) in May 2023 using the Geneious Alignment tool. Substitution models and evolutionary parameters for phylogenetic and selective pressure analyses were determined in MEGA X v10.0.5 [27]. The phylogenetic tree was reconstructed using the Maximum Likelihood method with the Tamura–Nei model (four parameters), incorporating a discrete gamma distribution, six invariable site categories, and 1000 bootstrap replications [28].

To evaluate the serological immune response, serum samples from November 2021 and February 2022 were tested by two in-house ELISAs, one targeting recombinant DCH core antigen (DCHcAg) and the other DCH surface antigen (DCHsAg) [8, 29]. Only IgG antibodies against DCHcAg were identified; both sera tested negative for IgM anti-DCHcAg and IgG anti-DCHsAg. No serum collected before November 2021 was available for retrospective viral or antibody testing. The cat was found deceased in May 2022 without visible traumatic injuries, and no necropsy could be conducted.

Results and Discussion

Since the first description of DCH in 2018, extensive research has aimed to clarify its distribution and pathogenic role in feline hepatic disease [1–4, 8–18]. Numerous reports have linked DCH infection with clinical symptoms and biochemical indicators of liver injury [2–4, 6, 10–17]. Cats carrying DCH frequently present elevated ALT levels [10, 15]. Furthermore, animals showing biochemical evidence of hepatic dysfunction are estimated to be three times more likely to test positive for DCH compared to cats with normal profiles [15]. Collectively, these data indicate that DCH may contribute to the onset or progression of feline hepatic disorders. Based on the clinical findings, diagnostic imaging, lab data, and virological analyses, the present case was defined as a chronic periportal hepatitis linked to DCH infection. The increase in ALT, ALP, GGT, bile acids, and bilirubin, along with leukogram changes, was compatible with lymphocytic cholangiohepatitis [30]. Histopathology confirmed moderate fibrosis, mild lymphoplasmacytic infiltration, and ductular hyperplasia within nearly all portal regions, consistent with periportal chronic hepatitis [31, 32].

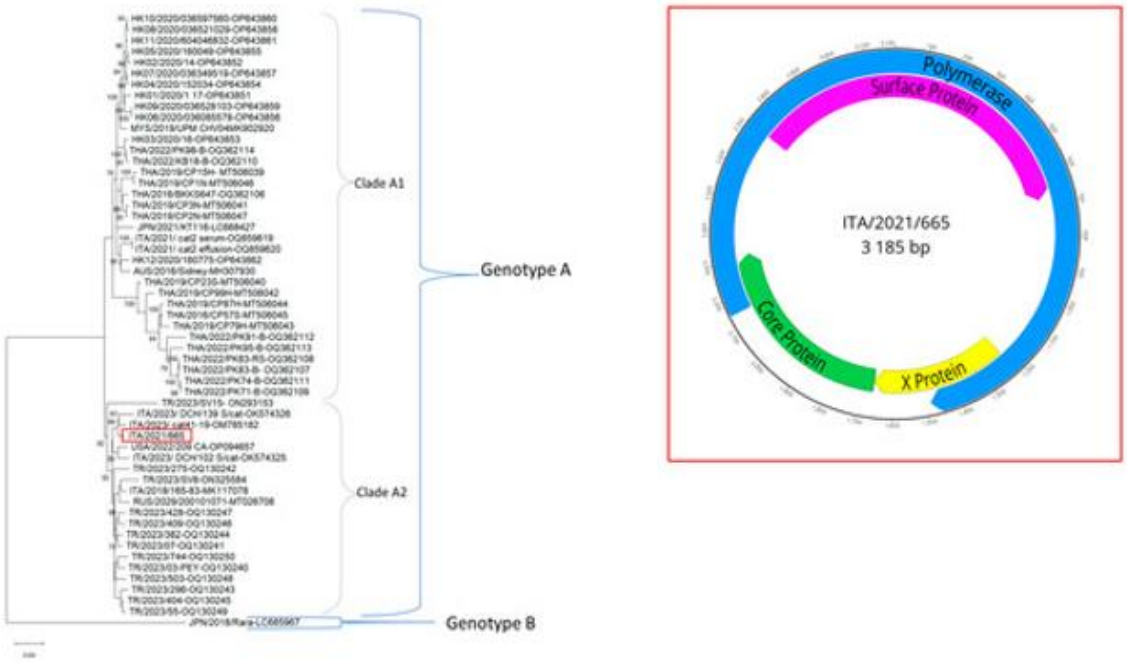
A retrospective study analyzing 71 feline liver biopsies identified a high rate of DCH in chronic hepatitis (43%) and hepatocellular carcinoma (28%), compared to histologically normal livers [9]. In DCH-positive chronic hepatitis cases, inflammation was predominantly lymphocytic at periportal zones and portal–lobular interfaces, with plasma cells interspersed through sinusoidal spaces. Neutrophils were rare and typically confined to necrotic foci or single-cell lesions. Fibrosis was unevenly distributed, extending through portal areas and affecting adjacent sinusoids [9]. More recently, Thai investigators reported a significant correlation between DCH presence and hepatic parenchymal disease (HPD), characterized by dense fibrotic bands bridging portal regions and extensive lymphoplasmacytic infiltration [11, 12].

In this case, IHC revealed cytoplasmic labeling for DCH that appeared multifocal and diffuse in hepatocytes and bile duct epithelial cells near inflamed or fibrotic areas. These findings align with those from the Thai study [12], which demonstrated DCH localization using polyclonal antibodies against HBcAg. In human HBV infections, immunohistochemical profiles for HBsAg and HBcAg correspond to viral replication activity and viral load, but this relationship is not yet established in cats. Consequently, the feline IHC results should be interpreted with caution due to unverified variability.

Interestingly, DCH DNA was detected in both liver and serum samples from November 2021, yet absent from the February 2022 serum, suggesting potential viral clearance or low-level persistence. In HBV-infected humans, similar phenomena occur in inactive carrier states or HBeAg-negative chronic hepatitis, often linked to genomic

mutations that render viral DNA minimally or non-detectable in blood [19, 33]. The cat in this case exhibited IgG anti-DCHcAg—a hallmark of chronic infection in HBV cases—but lacked anti-DCHsAg seroconversion, which in HBV typically signifies protective immunity and recovery [19, 33]. Both sera were also negative for IgM anti-core, excluding an acute or early infection phase. Integrating the serologic and molecular findings, the infection in this animal was best interpreted as chronic DCH infection.

The complete viral genome was 3185 bp in length and was submitted to GenBank (accession number OR389995) **(Figure 3)**.



a) b)

Figure 3. (a) Phylogenetic tree derived from full-length genomes of domestic cat hepadnavirus, including the isolate from this investigation (marked with a red square) and additional sequences retrieved from international databases. The robustness of branching was tested through 1000 bootstrap iterations, and only bootstrap scores above 75% are indicated. Genotypes a and b are labeled, with internal divisions A1 and A2 specified within genotype A. The scale bar denotes substitutions per nucleotide site. (b) Schematic layout of the DCH genome.

When examined across the entire genome, the DCH variant demonstrated a nucleotide similarity ranging between 97% and 98% with other Italian isolates [8, 10, 18]. In the phylogenetic reconstruction **(Figure 3)**, the full sequence of strain ITA/2021/665 grouped with Malaysian, Thai, Australian, European, and North American isolates under genotype A. This particular strain was positioned within a clearly separated lineage, designated clade A2, together with viruses from Italy, Russia, Turkey, and the United States **(Figure 3)**. Clade A2 was distinct from clade A1, which encompassed strains originating from Malaysia, Thailand, and Australia, as well as the Japanese Rara strain belonging to genotype B.

Conclusion

In conclusion, this report documents a feline case exhibiting biochemical and clinical alterations characteristic of liver impairment associated with long-term DCH infection. Evidence supporting this conclusion was derived from abdominal ultrasound findings, microscopic and immunohistochemical analysis of liver tissue, consistent abnormalities in hepatic enzyme markers, and detection of IgG anti-DCHcAg antibodies. Expanding the current understanding of DCH remains essential for clarifying its role in feline pathology and for creating effective diagnostic and preventive measures. Given that chronic HBV infection in humans can progress through fluctuating phases of viral replication, including intervals of low or undetectable viral load, a combined evaluation of clinical, biochemical, molecular, and serologic indicators is advised as the most reliable diagnostic strategy for DCH.

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Conflict of Interest: None

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Ethics Statement: None

References

1. Aghazadeh M, Shi M, Barrs VR, McLuckie AJ, Lindsay SA, Jameson B, et al. A novel hepadnavirus identified in an immunocompromised domestic cat in Australia. Viruses. 2018;10(5):269. doi:10.3390/v10050269
2. Capozza P, Carrai M, Choi YR, Tu T, Nekouei O, Lanave G, et al. Domestic Cat Hepadnavirus: molecular epidemiology and phylogeny in cats in Hong Kong. Viruses. 2023;15(1):150. doi:10.3390/v15010150
3. Shofa M, Kaneko Y, Takahashi K, Okabayashi T, Saito A. Global prevalence of domestic cat hepadnavirus: an emerging threat to cats' health? Front Microbiol. 2022;13:938154. doi:10.3389/fmicb.2022.938154
4. Sakamoto H, Ito G, Goto-Koshino Y, Sakamoto M, Nishimura R, Momoi Y. Detection of domestic cat hepadnavirus by next-generation sequencing and epidemiological survey in Japan. J Vet Med Sci. 2023;85(6):642-6.
5. Ratti G, Stranieri A, Scavone D, Cafiso A, Meazzi S, Luzzago C, et al. Detection and genetic characterization of domestic cat hepadnavirus in cats with cavitary effusions. Vet Microbiol. 2023;284:109828.
6. Capozza P, Decaro N, Beikpour F, Buonavoglia C, Martella V. Emerging hepatotropic viruses in cats: a brief review. Viruses. 2021;13(6):1162.
7. Adiguzel E, Erdem-Sahinkesen E, Koc BT, Demirden C, Oguzoglu TC. The detection and full genomic characterization of domestic cat orthohepadnaviruses from Türkiye. Vet Med Sci. 2023;9(8):1965-72.
8. Fruci P, Di Profio F, Palombieri A, Massirio I, Lanave G, Diakoudi G, et al. Detection of antibodies against domestic cat hepadnavirus using baculovirus-expressed core protein. Transbound Emerg Dis. 2022;69(6):2980-6.
9. Pesavento PA, Jackson K, Hampson T, Munday JS, Barrs VR, Beatty JA. A novel hepadnavirus is associated with chronic hepatitis and hepatocellular carcinoma in cats. Viruses. 2019;11(10):969.
10. Lanave G, Capozza P, Diakoudi G, Catella C, Catucci L, Ghergo P, et al. Identification of hepadnavirus in the sera of cats. Sci Rep. 2019;9(1):10668.
11. Piewbang C, Dankaona W, Poonsin P, Yostawonkul J, Lacharoje S, Sirivisoot S, et al. Domestic cat hepadnavirus associated with hepatopathy in cats: a retrospective study. J Vet Intern Med. 2022;36(5):1648-59.
12. Piewbang C, Wardhani SW, Chaiyasak S, Yostawonkul J, Chai-In P, Boonrungsiman S, et al. Insights into the genetic diversity, recombination, and systemic infections with evidence of intracellular maturation of hepadnavirus in cats. PLoS ONE. 2020;15(6):e0241212.
13. Stone C, Petch R, Gagne RB, Nehring M, Tu T, Beatty JA, et al. Prevalence and genomic sequence analysis of domestic cat hepadnavirus in the United States. Viruses. 2022;14(10):2091. doi:10.3390/v14102091
14. Takahashi K, Kaneko Y, Shibanai A, Yamamoto S, Katagiri A, Osuga T, et al. Identification of domestic cat hepadnavirus from a cat blood sample in Japan. J Vet Med Sci. 2022;84(5):648-52. doi:10.1292/jvms.22-0010
15. Anpuanandam K, Selvarajah GT, Choy MMK, Ng SW, Kumar K, Ali RM, et al. Molecular detection and characterisation of Domestic Cat Hepadnavirus (DCH) from blood and liver tissues of cats in Malaysia. BMC Vet Res. 2021;17(1):9. doi:10.1186/s12917-020-02700-0
16. Jeanes EC, Wegg ML, Mitchell JA, Priestnall SL, Fleming L, Dawson C. Comparison of the prevalence of Domestic Cat Hepadnavirus in a population of cats with uveitis and in a healthy blood donor cat population in the United Kingdom. Vet Ophthalmol. 2022;25(2):165-72.
17. Capozza P, Lanave G, Diakoudi G, Stasi F, Ghergo P, Ricci D, et al. A longitudinal observational study in two cats naturally-infected with hepadnavirus. Vet Microbiol. 2021;254:108999.

18. Scavone D, Lauzi S, Stranieri A, Tramontano G, Ratti G, Paltrinieri S. Evaluating the presence of domestic cat hepadnavirus viraemia in cats with biochemical alterations suggestive of liver disease. Vet Rec. 2022;191(10):e1626.
19. Seeger C, Zoulim F, Mason WS. Hepadnaviruses. In: Knipe DM, editor. Fields Virology. 7th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2013. p. 2185-210.
20. Lee JH, Kim HS. Current laboratory tests for diagnosis of hepatitis B virus infection. Int J Clin Pract. 2021;75(4):e14812.
21. Marsilio F, Di Martino B, Decaro N, Buonavoglia C. A novel nested PCR for the diagnosis of calicivirus infections in the cat. Vet Microbiol. 2005;105(1):1-7.
22. Decaro N, Desario C, Lucente MS, Amorisco F, Campolo M, Elia G, et al. Specific identification of feline panleukopenia virus and its rapid differentiation from canine parvoviruses using minor groove binder probes. J Virol Methods. 2008;147(1):67-71.
23. Stiles J, Bienzle D, Render JA, Buyukmihci NC, Johnson EC. Use of nested polymerase chain reaction (PCR) for detection of retroviruses from formalin-fixed, paraffin-embedded uveal melanomas in cats. Vet Ophthalmol. 1999;2(2):113-6.
24. Vogtlin A, Fraefel C, Albini S, Leutenegger CM, Schraner E, Spiess B, et al. Quantification of feline herpesvirus 1 DNA in ocular fluid samples of clinically diseased cats by real-time TaqMan PCR. J Clin Microbiol. 2002;40(2):519-23.
25. Gut M, Leutenegger CM, Huder JB, Pedersen NC, Lutz H. One-tube fluorogenic reverse transcription-polymerase chain reaction for the quantitation of feline coronaviruses. J Virol Methods. 1999;77(1):37-46.
26. Quackenbush SL, Dean GA, Mullins JI, Hoover EA. Analysis of FeLV-FAIDS provirus burden and productive infection in lymphocyte subsets in vivo. Virology. 1996;223(1):1-9.
27. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(6):1547-9.
28. Hasegawa M, Kishino H, Yano T. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol. 1985;22(2):160-74.
29. Fruci P, Palombieri A, Sarchese V, Aste G, Friedrich KG, Martella V, et al. Serological and molecular survey on domestic dog hepadnavirus in household dogs, Italy. Animals (Basel). 2023;13(4):729.
30. Jaffey JA. Feline cholangitis/cholangiohepatitis complex. J Small Anim Pract. 2022;63(12):573-89.
31. Weiss DJ, Armstrong PJ, Gagne J. Inflammatory liver disease. Semin Vet Med Surg Small Anim. 1997;12(1):22-7.
32. Fuentealba IC, Aburto EM. Animal models of copper-associated liver disease. Comp Hepatol. 2003;2(1):5.
33. Coffin CS, Zhou K, Terrault NA. New and old biomarkers for diagnosis and management of chronic hepatitis B virus infection. Gastroenterology. 2019;156(2):355-68.