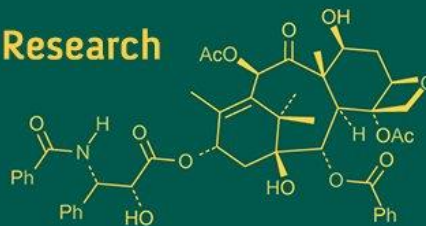
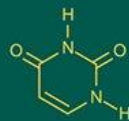


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Molecular screening of the ABCB11 gene and oxidative stress in canine liver disease

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Abstract

Liver disease is a common cause of morbidity in dogs and understanding its molecular basis may improve diagnosis and disease characterization. The present study investigated canine ABCB11 gene together with serum biochemical and oxidative stress biomarkers in dogs with naturally occurring liver disease. Thirty dogs comprising 10 healthy controls and 20 dogs diagnosed with liver disease were included in the study. Serum biochemical and oxidative stress parameters were estimated using standard spectrophotometric methods. A 577 bp fragment of ABCB11 gene was amplified by conventional polymerase chain reaction (PCR) using gene-specific primers. Dogs with liver disease showed significantly ($P < 0.05$) higher serum activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin concentrations, whereas total protein and albumin levels were significantly reduced compared with healthy dogs. A significant increase in serum MDA concentration and decrease in catalase activity in diseased animals indicate the oxidative stress. Conventional PCR successfully amplified the expected 577 bp ABCB11 gene fragment in all samples without any amplicon size differences between healthy and diseased dogs, indicating the absence of gross structural variation within the amplified region. The findings demonstrate that canine liver disease is associated with marked biochemical alterations and oxidative stress, while major structural changes in the targeted ABCB11 gene fragment were not detected. This study provides preliminary molecular information on the canine ABCB11 gene and supports the use of conventional PCR as an initial screening approach in investigations of canine hepatobiliary disorders.

Keywords: ABCB11, bile salt export pump, canine liver disease, oxidative stress, catalase, malondialdehyde

1. Introduction

Liver diseases are among the most common disorders encountered in canine clinical practice and represent a significant cause of morbidity and mortality worldwide. These disorders include inflammatory, infectious, metabolic, toxic, congenital and neoplastic origin that may lead to hepatocellular injury, cholestasis, fibrosis and progressive hepatic dysfunction [1-3]. Due to the important role of the liver in metabolic processes, detoxification, protein formation and bile secretion, the liver is vital in maintaining overall homeostasis. Therefore, any difficulty with the normal functioning of the liver can be evident through various diseases, needs early diagnosis and research into their pathogenesis.

Serum biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, albumin and total protein, are routinely used to evaluate liver injury and hepatic function in dogs [2, 4]. Oxidative stress also plays an important role in the progression of liver disease through excessive production of reactive oxygen species and depletion of antioxidant defenses. Malondialdehyde (MDA) and catalase are commonly used biomarkers of oxidative damage and their alterations have been reported in canine hepatic disorders [5-7]. Though these blood and oxidative biomarkers are important for assessing disease severity but they provide inadequate information regarding the molecular events underlying hepatobiliary disorders. Hence there is rising concern in identifying genes associated with bile acid transport and hepatocellular homeostasis that may contribute to the development and progression of liver disease.

The bile salt export pump (BSEP) is encoded by gene, ATP-binding cassette subfamily B member 11 (ABCB11), is an ATP-dependent transporter located on the canalicular

membrane of hepatocytes. BSEP plays an important role in the secretion of bile acids into the biliary canaliculi and is essential for maintaining normal bile acid homeostasis [8, 9]. Impaired BSEP function results in intracellular accumulation of bile acids, leading to cholestasis, inflammation, oxidative injury and progressive hepatocellular damage [9, 10]. In human medicine, mutations or altered expression of ABCB11 have been associated with several cholestatic disorders, including progressive familial intrahepatic cholestasis type 2, benign recurrent intrahepatic cholestasis and drug-induced cholestatic liver disease [10-12]. However, information regarding the molecular status of ABCB11 in naturally occurring canine liver disease remains limited.

Conventional polymerase chain reaction (PCR) is a reliable molecular technique for screening specific genomic regions and detecting major structural variations within amplified DNA fragments. Although conventional PCR cannot identify sequence-level mutations or evaluate gene expression, it provides useful preliminary information on the structural integrity of candidate genes and serves as an effective initial molecular screening tool [13]. Considering the limited information available on the molecular evaluation of the ABCB11 gene in dogs, the present study was undertaken to screen a targeted region of the canine ABCB11 gene using conventional PCR in dogs with naturally occurring liver disease. In addition, serum biochemical parameters and oxidative stress biomarkers were evaluated to examine their association with hepatic dysfunction. It was hypothesized that dogs with liver disease would exhibit significant biochemical and oxidative stress alterations, whereas major structural changes within the amplified region of the ABCB11 gene would not be detected.

2. Material and Method

2.1 Experimental animals

Thirty dogs of different breeds, ages and either sex were included in the study. Animals were selected on the basis of clinical examination, biochemical evaluation and history. The dogs were classified into two groups. Ten (10) apparently healthy dogs without any history or clinical evidence of hepatic or systemic disease served as healthy controls (Group-I). Twenty (20) dogs clinically diagnosed with naturally occurring liver disease were included in the Liver disease group (Group - II). Diagnosis was based on the presence of one or more clinical signs including jaundice, anorexia, lethargy, vomiting, abdominal discomfort, polyuria, polydipsia together with biochemical evidence of hepatic dysfunction characterized by increased serum ALT, AST, ALP and bilirubin concentrations. Dogs with severe renal disease, endocrine disorders, neoplasia or systemic illnesses unrelated to the liver were excluded to minimize confounding effects.

2.2 Blood sample collection

Approximately 5 mL of venous blood was collected aseptically from the jugular or cephalic vein using sterile disposable syringes. Each sample was divided into two portions. The first portion was transferred into EDTA-coated vacutainer tubes for genomic DNA extraction. The second portion was collected into plain vacutainer tubes for serum separation. Blood samples were allowed to clot at room temperature and centrifuged at 3000 rpm for 10 minutes. Separated serum was aliquoted into sterile microcentrifuge

tubes and stored at -20°C until biochemical and oxidative stress analyses.

2.3 Serum biochemical analysis

Serum biochemical analyses were performed using commercially available reagent kits (Erba Diagnostics Mannheim GmbH, Germany) according to the manufacturer's instructions. Measurements were carried out using a semi-automated biochemical analyser (Erba Chem-5X).

The biochemical parameters were determined were Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total protein, Albumin, Total Bilirubin, Direct Bilirubin. Serum globulin concentration was calculated by subtracting albumin concentration from total protein. These biochemical parameters were used to evaluate hepatocellular injury, cholestasis and hepatic synthetic function [2, 4].

2.4 Determination of oxidative stress biomarkers

Oxidative stress status was assessed by estimating serum catalase activity and malondialdehyde (MDA) concentration.

2.4.1 Catalase assay

Catalase activity was estimated using the spectrophotometric method described by Goth (14). Briefly, serum was incubated with hydrogen peroxide substrate for one minute at room temperature. The enzymatic reaction was terminated by adding ammonium molybdate solution, resulting in formation of a yellow chromogen. Absorbance was measured at 405 nm using a UV-visible spectrophotometer. Catalase activity was expressed as U/mL serum.

2.5.2 Malondialdehyde estimation

Serum malondialdehyde concentration was determined using the thiobarbituric acid reactive substances (TBARS) assay described by Suleiman (15). Briefly, serum samples were mixed with thiobarbituric acid-trichloroacetic acid reagent and heated in a boiling water bath for 15 minutes. Following cooling and centrifugation, absorbance of the supernatant was recorded at 532 nm. MDA concentration was calculated using the molar extinction coefficient of the MDA-TBA complex and expressed as nmol/mL serum.

2.5 Genomic DNA extraction

Genomic DNA was extracted from EDTA-treated whole blood using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and purity were determined using a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). DNA samples exhibiting an A260/A280 ratio between 1.80 and 2.00 were considered suitable for downstream PCR amplification.

2.6 Primer design

The canine ABCB11 gene sequence was retrieved from the NCBI GenBank database (Reference sequence XM_072779982.1). Primers were designed using Primer3 software. The primer sequence for forward primer 5'-CCATCCCCATCTCCCATTTGG -3' and that of reverse primer was 5'-CTTGTAGGGTCTGTGCTGCA-3'. Primer specificity was verified using NCBI Primer-BLAST to ensure amplification of the intended genomic region while minimizing non-specific amplification [16, 17]. The selected

primer pair amplified a 577 bp fragment of the canine ABCB11 gene.

2.7 Polymerase chain reaction (PCR)

PCR amplification was performed in a 20 µL reaction mixture containing 10 µL DreamTaq™ 2X PCR Master Mix (Takara Bio Inc., Japan), 0.5 µL Forward primer (10 µM), 0.5 µL Reverse primer (10 µM), 3 µL genomic DNA (approximately 50-100 ng), 6 µL nuclease-free water. Amplification was carried out in a programmable thermal cycler with the thermal cycling conditions as Initial denaturation: 94°C for 5 min, 32 cycles of: Denaturation: 94°C for 30 s, Annealing: 54°C for 30 s, Extension: 72°C for 45 s, Final extension: 72°C for 10 min, Hold at 4°C. Each PCR run included a no-template control (NTC) to monitor contamination.

2.8 Agarose gel electrophoresis: PCR products were electrophoresed on 1.5% agarose gel prepared in 1X Tris-Acetate-EDTA (TAE) buffer containing ethidium bromide (0.5 µg/mL). Electrophoresis was carried out at 90-100 V for approximately 45-60 minutes. A 100 bp DNA ladder (Thermo Fisher Scientific, USA) was used as the molecular weight marker. Amplified DNA fragments were visualized with Gel Documentation System and photographed for analysis. The presence of a single 577 bp amplification product was considered as successful amplification of the targeted ABCB11 gene fragment.

2.9 Statistical analysis: Data were analyzed using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA). Data distribution was evaluated using the Shapiro-Wilk normality test. Results are expressed as mean ± standard error of the mean (SEM). Comparisons between healthy dogs and dogs with liver disease were performed using an unpaired Student's t-test at significance level of $P < 0.05$.

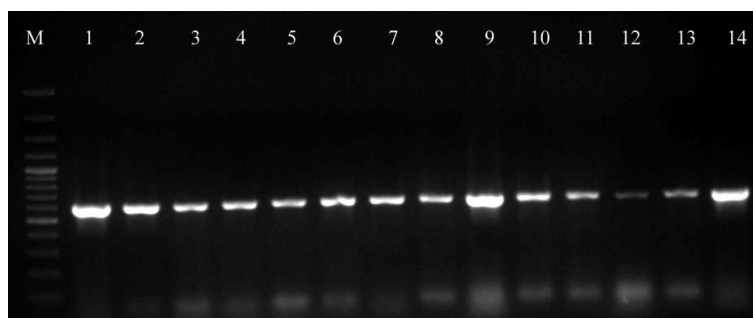


Fig 1: PCR amplification of the canine ABCB11 gene. Lane M: 100 bp DNA ladder; lanes 1-6: healthy control dogs; lanes 7-14: dogs with liver disease. A single amplification product of approximately 577 bp was detected in all samples.

3.3 Serum biochemical profile

Serum biochemical analysis showed significant variations in hepatic biomarkers in dogs affected with liver disease compared with clinically healthy controls (Table 1). Activities of serum ALT, AST and ALP were significantly higher ($P < 0.05$) in the diseased group. Mean ALT activity increased from 41.35 ± 1.62 IU/L in healthy dogs to 144.40 ± 2.13 IU/L in diseased animals, representing an approximately 3.5-fold increase. Similarly, AST activity increased from 30.94 ± 1.91 IU/L to 126.70 ± 2.12 IU/L, while ALP activity increased from 38.47 ± 2.20 IU/L to 263.50 ± 4.79 IU/L, corresponding to an approximately 6.8-fold rise. Serum bilirubin concentration was also significantly different between the two groups. Total bilirubin increased

3. Results

3.1 Clinical observations

Dogs included in the liver disease group showed a wide range of clinical symptom consistent with hepatobiliary dysfunction. The most frequently observed clinical signs were anorexia, lethargy, jaundice, vomiting, dehydration, abdominal discomfort and weight loss. Additionally, some animals had pale to jaundiced mucus membranes, poor appetite, polyuria, polydipsia and occasional diarrhea. In advanced cases, the presence of ascites was evident, accompanied by some neurological disorders associated with hepatic encephalopathy. On the other hand, no signs of hepatic or systemic disease were noted in any of the healthy dogs used as controls.

3.2 PCR amplification of the canine ABCB11 gene

High-quality genomic DNA was successfully isolated from all blood samples. The A260/A280 ratio was ranged between 1.80 to 2.00 showed purity of extracted DNA and its suitability for PCR amplification. Conventional PCR successfully amplified the targeted region of the canine ABCB11 gene in all samples included in the study. Following electrophoresis on 1.5% agarose gel, each sample produced a distinct amplification product corresponding to the expected 577 bp fragment (Figure 1). No additional amplicon or non-specific bands were observed. The same PCR amplification pattern was observed between the normal dogs and those with liver diseases. The size of the amplification product and their electrophoretic pattern of migration did not differ in either group. These findings indicate that major insertions, deletions, or gross structural alterations were not detected within the amplified region of the canine ABCB11 gene evaluated in the present investigation.

significantly from 0.39 ± 0.02 mg/dL in healthy dogs to 2.98 ± 0.07 mg/dL in dogs with liver disease. Likewise, direct bilirubin concentration increased from 0.19 ± 0.01 mg/dL to 1.81 ± 0.09 mg/dL ($P < 0.05$). On the other hand, serum proteins showed significant reductions in liver disease group. Total protein decreased from 6.77 ± 0.32 g/dL to 5.07 ± 0.19 g/dL, whereas albumin concentration declined from 3.48 ± 0.19 g/dL to 2.49 ± 0.10 g/dL. Similarly, globulin concentration decreased significantly from 3.39 ± 0.36 g/dL in healthy dogs to 2.59 ± 0.21 g/dL in diseased dogs. These biochemical findings collectively indicate marked hepatocellular injury, cholestasis and impaired hepatic synthetic function.

Table 1. Serum biochemical parameters in healthy dogs and dogs with liver disease

Parameter	Unit	Healthy Dogs (n = 10)	Diseased Dogs (n = 20)	Fold change
Total Serum Protein	g/dL	6.77 ± 0.32	5.07 ± 0.19*	↓1.34
Serum Albumin	g/dL	3.48 ± 0.19	2.49 ± 0.10*	↓1.40
Serum Globulin	g/dL	3.39 ± 0.36	2.59 ± 0.21*	↓1.31
AST	IU/L	30.94 ± 1.91	126.7 ± 2.12*	↑4.09
ALT	IU/L	41.35 ± 1.62	144.4 ± 2.13*	↑3.49
ALP	IU/L	38.47 ± 2.20	263.5 ± 4.79*	↑6.85
Total Bilirubin	mg/dL	0.39 ± 0.02	2.98 ± 0.07*	↑7.64
Direct Bilirubin	mg/dL	0.19 ± 0.01	1.81 ± 0.09*	↑9.53
Catalase	U/mL	45.25 ± 0.87	25.4 ± 1.01*	↓1.78
MDA	nmol/mL	2.41 ± 0.11	7.01 ± 0.27*	↑2.91

3.4 Oxidative stress biomarkers

Evaluation of oxidative stress parameters showed that dogs with liver disease had a significant difference in redox equilibrium (Table 2). Serum MDA concentration increased significantly ($P < 0.05$) in affected dogs. Healthy animals exhibited an average MDA concentration of 2.41 ± 0.11 nmol/mL, whereas diseased animals showed 7.01 ± 0.27 nmol/mL, representing an approximately 2.9-fold increase. Whereas, catalase activity was significantly lower in dogs with liver disease. Mean catalase activity decreased from 45.25 ± 0.87 U/mL in healthy dogs to 25.40 ± 1.01 U/mL, corresponding to an approximate 54% reduction. The simultaneous increase in MDA together with reduced catalase activity indicates enhanced lipid peroxidation accompanied by depletion of endogenous antioxidant defenses.

4. Discussion

The present study explored a targeted region of the ABCB11 gene along with serum biochemical and oxidative stress biomarkers in dogs with naturally occurring liver disease. Conventional PCR successfully amplified the expected 577 bp fragment of the ABCB11 gene in all samples and no differences in amplicon size were detected between healthy and diseased dogs. The dogs with liver disease exhibited obvious biochemical indication of hepatocellular injury and cholestasis, accompanied with significant oxidative stress.

The significant increases in serum ALT, AST and ALP activities observed in presented diseased dogs are consistent with previous reports describing hepatic injury in canine liver disorders [2, 4, 18]. ALT is considered the most sensitive marker of hepatocellular damage because of its cytosolic localization in hepatocytes, whereas AST reflects more extensive cellular injury involving both cytoplasmic and mitochondrial compartments (2). The increase in ALP, together with elevated total and direct bilirubin concentrations, indicates impaired bile flow and cholestatic dysfunction. These biochemical changes are characteristic of chronic hepatitis, cholangitis and other hepatobiliary disorders reported in dogs [18, 19]. Significant reductions in serum total protein, albumin and globulin concentrations were also observed in affected animals. Albumin is synthesized exclusively by hepatocytes and decreased serum albumin generally reflects impaired hepatic synthetic function, particularly in chronic or advanced liver disease [2, 4]. Reduced protein synthesis consequential from progressive hepatocellular damage may related with the lower serum protein concentrations observed in the diseased group. Similar findings have been reported in canine chronic hepatitis and cirrhosis [18].

A major objective of this study was to evaluate whether naturally occurring canine liver disease is associated with

detectable structural alterations in the ABCB11 gene. The uniform amplification of the expected 577 bp fragment in all samples and the absence of differences in amplicon size indicate that major insertions, deletions, or other gross structural changes were not present within the amplified genomic region. This finding suggests that large genomic alterations in this portion of the ABCB11 gene are unlikely to be a common cause of liver disease in the studied dogs. However, the absence of structural variation in the PCR product should not be interpreted as evidence that ABCB11 has no role in canine liver disease. Conventional PCR is capable of detecting only major changes within the amplified fragment and cannot identify single nucleotide polymorphisms, small insertions or deletions, promoter variants, or alterations in gene expression [13]. Therefore, slight genetic variants or transcriptional dysregulation of ABCB11 may still contribute to impaired bile acid transport. In human medicine, mutations and altered expression of ABCB11 have been associated with progressive familial intrahepatic cholestasis, drug-induced cholestasis and other cholestatic disorders [8, 12]. Whether similar mechanisms operate in dogs requires further investigation using DNA sequencing and gene expression analyses.

Dogs with liver disease showed significantly higher serum MDA concentrations together with reduced catalase activity, indicating increased lipid peroxidation and decreased antioxidant defense. MDA is a well-established marker of oxidative damage to cell membranes, whereas catalase is crucial for detoxifying hydrogen peroxide and preventing oxidative injury [5-7]. The observed increase in MDA and decrease in catalase activity are consistent with previous studies demonstrating oxidative imbalance in canine hepatic disorders. The association between oxidative stress and bile acid transport is important. Accumulation of bile acids within hepatocytes can promote mitochondrial dysfunction, reactive oxygen species generation and inflammatory signaling, thereby exacerbating hepatic injury [5, 9]. further, oxidative stress may affect the expression and function of bile acid transporters, including BSEP, through regulatory rather than structural mechanisms. Although the present study did not evaluate gene transcription or translation, the result presents grounds for future studies investigating functional regulation of ABCB11 in canine liver disease. This study has certain limitations. The sample size was relatively small and only a single region of the ABCB11 gene was examined using conventional PCR. Therefore, sequence-level mutations and changes in gene expression could not be assessed. Future studies should incorporate complete gene sequencing, quantitative expression analysis and functional assessment of BSEP to better define the role of ABCB11 in canine hepatobiliary disorders.

5. Conclusions

The present work is on preliminary molecular assessment of the ABCB11 gene in dogs with naturally occurring liver disease using conventional PCR. Successful amplification of the targeted 577 bp fragment was attained in all samples and no differences in PCR amplicon size were observed between healthy and diseased dogs. These findings showed that no gross structural variation was detected within the amplified region of the ABCB11 gene in the study population. The dogs with liver disease showed significant changes in serum biochemical parameters, including elevated activities of ALT, AST, ALP and increased bilirubin concentrations, together with reduced total protein and albumin levels, corroborated the hepatocellular injury and impaired hepatic function. Furthermore, the significant increase in serum malondialdehyde and reduced catalase activity demonstrated the presence of oxidative stress, highlighting its important role in the patho-physiology of canine liver disease. Although there were no significant structural alterations observed in the amplified ABCB11 gene segment, the result is a source of molecular data for the this gene in canine liver disorders. In order to get a better understanding of the importance of ABCB11 in canine hepatobiliary disorders, further studies that involve larger samples and more methods for gene expression evaluation are required.

References

- Watson PJ. Chronic hepatitis in dogs: a review of current understanding of the aetiology, progression, and treatment. *Vet J*. 2004 May;167(3):228-241.
- Center SA. Interpretation of Liver Enzymes. *Vet Clin North Am Small Anim Pract*. 2007 Mar;37(2):297-333.
- Cullen JM, Stalker MJ. Liver and Biliary System. In: Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 2. Elsevier; 2016. p. 258-352.e1.
- Lawrence YA, Steiner JM. Laboratory Evaluation of the Liver. *Vet Clin North Am Small Anim Pract*. 2017 May;47(3):539-553.
- Webb C, Twedt D. Oxidative Stress and Liver Disease. *Vet Clin North Am Small Anim Pract*. 2008 Jan;38(1):125-135.
- Blanca P-M, María Luisa F-R, Guadalupe M, Fátima C-L. Oxidative Stress in Canine Diseases: A Comprehensive Review. *Antioxidants*. 2024 Nov 15;13(11):1396.
- Sangamitra RKR, Suganya G, Leela V, Balagangadhara Thilagar M. Evaluation of oxidative stress in dogs with hepatic dysfunction. *Indian J Vet Anim Sci Res*. 2021;50(4):47-53.
- Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, *et al*. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. *Nat Genet*. 1998 Nov;20(3):233-238.
- Stieger B, Meier Y, Meier PJ. The bile salt export pump. *Pflügers Arch - Eur J Physiol*. 2007 Jan 29;453(5):611-620.
- Jansen PLM, Strautnieks SS, Jacquemin E, Hadchouel M, Sokal EM, Hooiveld GJEJ, *et al*. Hepatocanalicular bile salt export pump deficiency in patients with progressive familial intrahepatic cholestasis. *Gastroenterology*. 1999 Dec;117(6):1370-1379.
- van Beusekom CD, van den Heuvel JJ, Koenderink JB, Schrickx JA, Russel FG. The feline bile salt export pump: a structural and functional comparison with canine and human Bsep/BSEP. *BMC Vet Res*. 2013 Dec 20;9(1):259.
- Zollner G. Hepatobiliary transporter expression in percutaneous liver biopsies of patients with cholestatic liver diseases. *Hepatology*. 2001 Mar;33(3):633-646.
- Russell DW, Sambrook J. Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor Laboratory Press; 2001.
- Góth L. A simple method for determination of serum catalase activity and revision of reference range. *Clin Chim Acta*. 1991 Feb;196(2-3):143-151.
- Suleiman SA, Ali ME, Zaki ZMS, El-Malik EMA, Nasr MA. Lipid Peroxidation and Human Sperm Motility: Protective Role of Vitamin E. *J Androl*. 1996 Sep 10;17(5):530-537.
- Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, *et al*. Primer3—new capabilities and interfaces. *Nucleic Acids Res*. 2012 Aug;40(15):e115-e115.
- Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*. 2012 Dec 18;13(1):134.
- Webster CRL, Center SA, Cullen JM, Penninck DG, Richter KP, Twedt DC, *et al*. ACVIM consensus statement on the diagnosis and treatment of chronic hepatitis in dogs. *J Vet Intern Med*. 2019 May 1;33(3):1173-1200.
- Kruitwagen HS, Spee B, Schotanus BA. Hepatic progenitor cells in canine and feline medicine: potential for regenerative strategies. *BMC Vet Res*. 2014;10(1):137.