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FULL PAPER

Pathology

Lobular diameters of autopsied dog livers give clues for an appropriate liver biopsy methodology

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ABSTRACT. Hepatobiliary diseases of animals are frequently diagnosed by a combination of imaging, clinical pathology, and histopathology. A standardized surgical liver biopsy protocol, however, has not been established in veterinary medicine with regard to the selection of lobe and site of the liver to yield the most diagnostic information. To address this matter, we histologically examined 33 livers of autopsied dogs from which tissue samples of 4 different lobes as well as 4 different sites of each lobe were prepared. We measured the hepatic lobular diameter (HLD) as an objective variable to refer to the inter-lobar or inter-site difference among the biopsied samples. A measurement of 2,623 hepatic lobules resulted in 1.042 mm as the average of all the HLD values. Statistical analysis further revealed that the HLD tended to be small in a superficial 2 mm area of the liver parenchyma regardless of biopsy location, thus this area should be evaluated carefully by pathologists. The results also suggest that the HLD values of the quadrate lobe may measure smaller than those in the other lobes. Therefore, one would be able to obtain representative data of the entire liver by taking a sample from any single lobe except for the quadrate lobe. HLD measurements are needed in order to accumulate potentially useful information on the microanatomy and pathophysiology of the liver.

KEY WORDS: autopsy, biopsy, diameter, dog, liver

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Spontaneous diseases of the hepatobiliary system of domestic animals are diverse and have both similarities and differences when compared to those of humans [8]. As an example of an animal-specific condition, there has been little research published in the literature dealing with primary portal vein hypoplasia (PPVH, alias hepatic microvascular dysplasia [6]) in humans. Briefly PPVH is mainly a canine (rarely a feline) congenital vascular disorder, that is recognized by an elevation of liver-related values such as alanine aminotransferase and bile acid, manifested by a decreased portal vein to aorta ratio on computed tomography (CT) imaging and various hepatic histologic alterations quite similar to those found in the liver of human and animal patients with a portosystemic shunt (PSS) [1, 9, 16, 20, 26, 27]. Though most animals with PPVH show mild or no clinical signs and only require modest medical treatment [1], this disease is not amenable to surgery and there have been rare cases that developed portal hypertension and ascites [27]. This seemingly indolent canine PPVH is gaining scientific attention regarding its application to the development of a hepatocyte growth factor treatment for more aggressive liver diseases of other types, such as chronic hepatitis or cirrhosis [22].

Histological examination has been the gold standard in diagnosing and categorizing human hepatic diseases [23], but biopsy of the liver is also occasionally performed in veterinary medicine, especially for companion animals such as dogs and cats [7]. To diagnose PPVH in dogs, liver tissue of a size larger than that produced by needle biopsy is required to assess the architectural integrity of hepatic lobules as well as multiple portal tracts in addition to non-invasive diagnostic modalities such as ultrasonography (US) or CT [27]. Fine-needle aspiration or the biopsy gun technique are unsuitable for the diagnosis of PPVH because one has to evaluate a change in the size and number of interlobular arteries and interlobular veins in multiple portal tracts [27]. Hepatocellular atrophy is also an important histologic feature of canine congenital hepatic vascular anomalies such as PPVH or PSS likely because of a decreased supply of nutrition and oxygen via the attenuated (extrahepatic) portal vein or by-passing of

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the liver by shunt vessels [1, 13]. Consequently, as long as the risks of anesthesia can be adequately managed, an excisional biopsy (partial hepatectomy) during laparotomy or laparoscopy, in spite of the requirement for general anesthesia, is preferred to the fine-needle aspiration or biopsy gun method because the former method can yield a larger sample which provides more information for the diagnosis of PPVH or PSS [27]. Excisional biopsy from the canine liver is also often performed during an elective surgery such as ovariohysterectomy or laparotomy for other purposes [7]. A standardized surgical liver biopsy protocol, however, is not fully established in veterinary medicine with regard to selection of lobe and site of the liver to yield the most diagnostic information.

Whether we should call the functional unit of the liver "liver lobule" or "liver acinus" seems to have been controversial [2, 18]. Though species difference is clearly present in hepatic microarchitecture [2, 8, 17, 27], much of the literature on humans [18] and animals [8] use common terminology to describe components of hepatic lobules/acini. Examples of these include the central veins, hepatic cords, sinusoids, and portal tracts [8, 18]. Portal tracts are further composed of interlobular/portal veins or hepatic venules, interlobular artery or hepatic arteries/arterioles, and bile ducts/ductules [8, 18]. Sub-gross or low-magnification evaluation of hepatic histology specimens has traditionally focused on the presence or absence as well as patterns or distribution of abnormality [24] without mentioning the size/diameter of hepatic lobules *per se*. The diameter of a hepatic lobule has been reported in the literature to be about 1 mm in humans [19] and 1 to 2 mm in animals [4], respectively, but without supporting references or evidence by the authors. Also, there are no reports investigating the hepatic lobular diameter (HLD) and standardized methods to measure it. Moreover, qualitative differences between various excisional biopsy techniques i.e. wedge biopsy from the lobar edge versus "scooping/spooning" biopsy from the lobar body have not been objectively examined.

The aims of our study are: 1) to determine the mean HLD value of dogs of varied disease status as the basis of future hepatobiliary investigation; 2) to examine the effect of topology and method of sampling on the measurement of HLD; 3) to establish an adequate methodology of post-sampling handling/trimming and histological evaluation of dog livers.

MATERIALS AND METHODS

Case selection

The initial sample pool included 90 client-owned canine cases whose autopsies were performed by two board-certified veterinary pathologists (Ikki Mitsui, Yoshio Kawamura) and 32 canine cases whose autopsies were performed by non-pathologist attending veterinarians with subsequent submission of formalin-fixed organ samples (so-called "autopsy in a jar") to No Boundaries Animal Pathology, LLC (formerly affiliated with Ikki Mitsui, Tokyo, Japan) from December 2012 to April 2018. All the autopsies were conducted after written or verbal informed consent had been obtained from the owners. Organs fixed in 10 or 20% neutral-buffered formalin for various periods (1 month to 5 years) were screened for preservation of all the hepatic lobes, complete absence of severe diffuse hepatic diseases such as cirrhosis, massive necrosis, or primary neoplasm, and absence of significant desiccation or liquefaction of the tissue due to prolonged storage. The detailed information on tissue processing is available in Supplementary Table 1. No type (i.e. extrahepatic or intrahepatic) of PSS was reported by the submitter in any of these cases. Eventually, 33 cases met the above criteria, 20 of which were autopsied by board-certified veterinary pathologists (case 1 to 20) and the rest (cases 21 to 33) were autopsied by non-pathologist attending veterinarians.

Tissue sampling and trimming

Dog liver is composed of 6 lobes, namely left lateral (LL), left medial, quadrate (Q), right medial (RM), right lateral (RL), and caudate lobes. Tissues were trimmed from four hepatic lobes (LL, Q, RM, RL) from each dog by a scalpel. The reason for the selection of these four hepatic lobes was their better visibility and accessibility during laparotomy or laparoscopy than the remaining lobes (left medial and caudate) that reside in a deep dorsal area of the cranial abdomen. Each sample set included four 2-mm-thick slices from different locations, depth, or plane, namely, the periphery of the lobe (E; edge) measuring approximately 10 × 10 mm, an approximately 10 × 10 mm plane vertical to the hepatic capsule in the body of the lobe (B; body), two approximately 10 × 10 mm planes parallel to the hepatic capsule in the lobar body excised at 2 mm (B2) and 4 mm (B4) deep from the capsular surface respectively. The trimming methodology is illustrated in Fig. 1. This method intended to simulate "wedge" biopsy from the lobar periphery (E) and "scooping/spooning" biopsy from the lobar body with different post-fixation trimming methods depending on the size, shape, and thickness of the specimen or variation in methodology of histology technicians in charge of trimming (B, B2, B4).

Histopathology

Each tissue sample was routinely processed, sectioned at 4 μm, and stained with hematoxylin and eosin (HE) for histopathology. A board-certified veterinary pathologist (Ikki Mitsui) examined all the histospecimens using a light microscope (ECLIPSE Ci, Nikon, Tokyo, Japan). Masson trichrome (MT) stain was performed for the duplicate sections in each case to facilitate measurement of the HLD and evaluate the degree of fibrosis. Due to prolonged fixation of most of the samples, immunohistochemistry or molecular techniques of any kind were not tried in this study.

Measurement of the HLD

A board-certified veterinary pathologist (Ikki Mitsui) examined all the histospecimens. A light microscope digital camera (DS-Fi2, Nikon) and a stand-alone histomorphometry unit (DS-L3, Nikon) were used throughout the examination. According to the general definition of hepatic lobule or acinus, the distance between the neighboring portal tracts or the distance between the

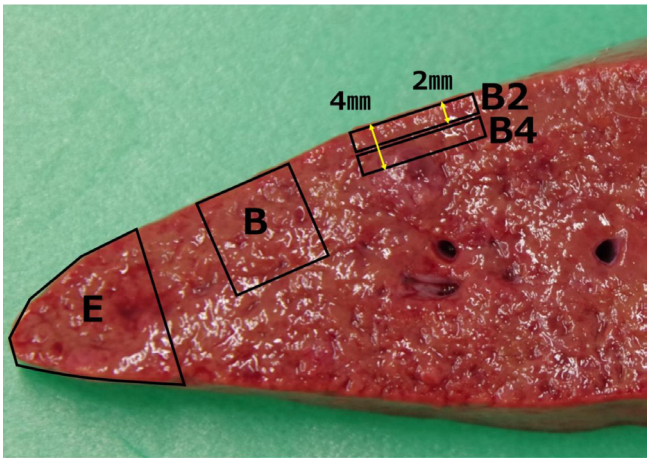

Fig. 1. Photograph detailing location, depth, or plane of each tissue section.

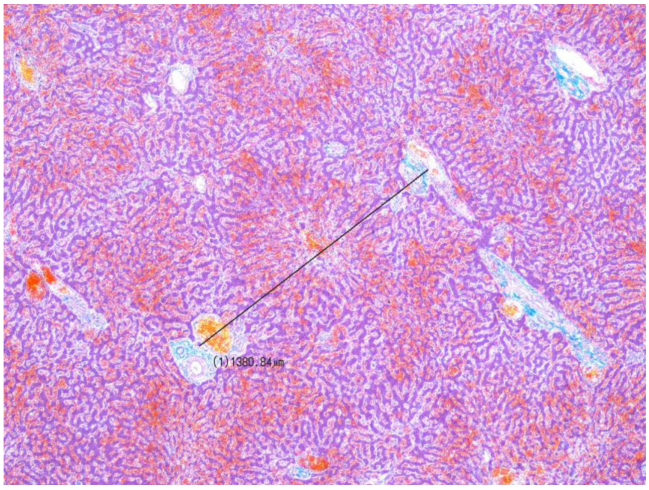

Fig. 2. Photomicrograph showing measurement of the hepatic lobular diameter using a histospecimen of the canine liver (case 18, left lateral lobe). Masson trichrome stain.

neighboring central veins were measured and recorded as HLD (Fig. 2). When measuring the distance between the neighboring portal tracts, the approximate center of each portal tract was chosen as a starting/ending point because of the random size and distribution of the components (e.g. interlobular vein, interlobular artery, and bile duct) within a given portal tract. The measurement was always performed at 40× magnification using light microscopy, with a combination of 10× ocular and 4× objective. Five measurements were performed for each tissue before application of statistics, thus, the total number of HLD measurements amounted to 2,640 (33 dogs × 4 different liver lobes × 4 different sampling locations × 5 measurements). As long as portal tracts were detectable without difficulty, the distance between the neighboring pair of portal tracts was measured preferentially over the distance between the neighboring central veins because the central veins varied more in diameter and degree of perivascular fibrosis than portal tracts. In cases of histospecimens of inadequate condition such as severe autolysis, measurements were not performed to avoid misinterpretation.

Statistical analysis

Five measurements of the HLD of each tissue were averaged and used for statistical analysis using a commercially available software (SPSS Statistics 23, IBM Corp., Armonk, NY, USA). Two assumptions were tested in this experiment: 1. Is there a significant difference among the HLD values of each sampling location (E, B, B2, B4) in a given hepatic lobe? 2. Is there a significant difference among the HLD values of each lobe (LL, Q, RM, RL) at a given sampling location? Shapiro-Wilk normality test, repeated measures analysis of variance (ANOVA), and Bonferroni multiple comparison tests were applied for these assumptions, using a statistical significance level of 0.05.

HLD measurement in biopsied liver samples from PPVH dogs

We measured HLD in wedge-biopsied liver specimens from five client-owned dogs (Table 4). These dogs were judged to have PPVH because they met the following selection criteria: 1) abnormally small liver (microhepatia) without renomegaly as detected by abdominal radiograph, ultrasound, and/or intraoperative gross observation; 2) absence of shunting blood vessels as confirmed by abdominal ultrasound and intraoperative gross observation; 3) detection of typical microscopic changes (bile duct proliferation, hypoplasia of interlobular veins, hepatocellular atrophy, and arteriolar proliferation or duplication) on HE-stained sections. HLD measurements were performed on 4-μm-thick sections stained with MT as previously written.

RESULTS

Signalment and clinicopathological information

Signalment and clinicopathological information of the 33 dogs (age, sex, breed, type of death, main gross lesions) are summarized in Table 1. Median age of the 33 dogs was 96 months (8 years) and mean age was 108.8 months (9 years and 0.8 month), respectively. Ratio of female to male was 14:19. There were 2 intact females (6.06%), 12 spayed females (36.36%), 8 intact males (24.24%), and 11 castrated males (33.33%). Miniature Dachshunds were overrepresented (7) and various, mainly small-breed dogs followed Miniature Dachshunds in descending order: Chihuahua (4), Toy Poodle (3), Shiba Inu (3), mixed breed (3), French Bulldog (2), Beagle (2), and one for each of Maltese, Miniature Schnauzer, Norfolk Terrier, Papillon, Pug, Shetland Sheepdog, Shih Tzu, Welsh Corgi, and Yorkshire Terrier.

Table 1. Summary of signalment and clinicopathological information of the 33 dogs

Case number	Age	Sex[a)]	Breed	Type of death[b)]	Main lesions[c)]
1	6y4m	CM	Toy Poodle	S	Hemoabdomen, hepatic capsular lysis, acute pancreatitis
2	7m	SF	French Bulldog	S	Hemoabdomen, hepatic capsular lysis, acute pancreatitis
3	6y10m	F	Miniature Dachshund	S	DAD
4	11y	SF	Chihuahua	S	DAD, pulmonary edema, mitral valvular myxomatous degeneration
5	5m	M	Chihuahua	S	Generalized hyperemia/congestion
6	2y	CM	Papillon	S	Healed skin trauma, hemoabdomen, rib fracture, multiple lacerations in the left lateral lobe of the liver
7	6y5m	CM	Toy Poodle	S	Severe acute pulmonary hemorrhage
8	13y7m	SF	Mix	S	Hemangiosarcoma in the right auricle
9	11y5m	M	Welsh Corgi	S	Hemangiosarcoma (spleen, liver, right atrium), hemoabdomen
10	11y9m	M	Shiba Inu	N	Alimentary T-cell LGL lymphoma, fungal gastritis, emaciation, interstitial pneumonia
11	4y	SF	Toy Poodle	S	Gastric impaction with "pill pocket", mural hemorrhage of the gastric wall, hyperemia of multiple organs
12	11y	CM	Miniature Dachshund	N	Prostatic carcinoma metastasized to the lung, multiple lymph nodes, bones, etc.
13	14y	SF	Yorkshire Terrier	N	Pulmonary edema, chronic renal failure, cardiac enlargement and focal myocardial necrosis, ascites, thoracic effusion
14	5y9m	CM	Mix	S	Bacterial necrotizing colitis, interstitial pneumonia
15	8y	SF	Miniature Dachshund	S	Severe deposition of dental tartar, pulmonary edema/acute interstitial pneumonia
16	5y	M	Shiba Inu	S	Alimentary lymphoma, multifocal thrombosis, pulmonary edema, likely disseminated intravascular coagulation
17	8y	CM	Shih Tzu	S	Acute pancreatic necrosis or postmortem change
18	15y	CM	Mix	N	Multiple endocrine neoplasia, multifocal vasculitis, arteriolosclerosis
19	11y5m	CM	Chihuahua	S	Mitral valvular myxomatous degeneration, bacterial pneumonia, cecal adenocarcinoma
20	5y6m	M	Shiba Inu	S	Hyperplastic catarrhal lymphoplasmacytic colitis with intralesional whipworms, acute pancreatitis
21	11y6m	M	Shetland Sheepdog	N	Pancreatitis (acute to chronic), intimal thickening of the coronary vessels, focal myocardial necrosis, cardiac vasculitis and valvulitis, glomerulonephritis (suspected), chronic interstitial nephritis
22	15y	M	Miniature Dachshund	N	Systemic metastasis of malignant melanoma, multiple fibrin thrombosis, chronic interstitial nephritis, suppurative prostatitis, etc.
23	6y8m	M	Norfolk Terrier	N	Multifocal thrombosis, mast cell tumor with vascular invasion and dissemination to anterior mediastinum, adrenals, multiple lymph nodes, liver, spleen, interstitial pneumonia, etc.
24	6y1m	CM	Miniature Schnauzer	S	Multifocal pulmonary hemorrhage and edema, intrahepatocellular cholestasis, hepatocellular degeneration and necrosis, cholemic nephrosis, acute tubular injury, hemorrhage and hyperemia/congestion in multiple organs
25	10y	F	Chihuahua	E	DAD, suppurative laryngotracheitis, cardiocellular hypertrophy, mitral valvular myxomatous degeneration
26	16y11m	SF	Maltese	N	Disseminated hemangiosarcoma possibly derived from the liver (kidney, heart, pancreas, lung, adrenals), chronic interstitial nephritis, severe adrenal atrophy
27	18y4m	CM	Beagle	S	Diffuse pulmonary edema, coronary vascular amyloid deposition, cardiocellular hypertrophy, scattered glomerular thrombosis, degeneration and necrosis of proximal tubules, metastasis of anal sac gland carcinoma to abdominal lymph node, etc.
28	14y2m	CM	Miniature Dachshund	N	Signet-ring carcinoma (likely of duodenal origin) with vascular invasion and dissemination, marked sclerosis of the omentum and duodenum, dilation of common bile duct
29	2y10m	SF	French Bulldog	S	Diaphragmatic hernia (hepatic lobar eventration), subserosal hemorrhage at the gastric greater curvature
30	12y	SF	Beagle	E	Hemangiosarcoma in the right auricle with metastasis/dissemination to the lung, liver, kidney, adrenals, peritoneum, pulmonary hemorrhage, DAD
31	7y6m	SF	Pug	N	Ileal large-cell lymphoma, gastritis, enteritis (except for the ileum), colitis
32	7y	SF	Miniature Dachshund	N	Pulmonary edema with bacterial colonization, cardiac hypertrophy, acute tubular injury, etc.
33	13y2m	SF	Miniature Dachshund	N	DAD, metastasis/dissemination of "mammary carcinoma and malignant myoepithelioma" to the lung, tracheobronchial lymph node, and acute renal tubular injury

a) CM=castrated male, M=intact male, SF=spayed female, F=intact female. b) S=sudden death, N=natural death, E=euthanasia. c) DAD=diffuse alveolar damage.

Table 2. Summary of the measured values in micrometers of the hepatic lobular diameter of 33 dogs

Liver lobe	Left lateral lobe				Quadrate lobe				Right medial lobe				Right lateral lobe			
Case number	E	B	B2	B4	E	B	B2	B4	E	B	B2	B4	E	B	B2	B4
1	849.0	731.2	918.6	1,053.6	977.4	844.4	930.4	968.2	858.6	819.0	909.8	777.0	772.6	673.6	908.2	935.6
2	838.0	997.2	967.6	1,025.6	996.4	1,103.0	1,259.8	1,267.6	1,061.8	1,296.8	1,540.8	1,423.4	1,415.4	1,558.2	1,379.0	1,301.4
3	1,401.4	1,365.0	1,121.0	1,372.8	1,128.8	1,069.6	1,050.2	1,078.8	879.4	1,102.8	889.6	858.0	908.8	866.4	879.2	1,026.6
4	1,001.8	1,085.6	1,009.8	1,094.0	967.8	815.2	954.8	*	859.6	960.8	1,023.0	1,054.8	875.0	1,024.8	1,123.4	1,205.0
5	934.0	861.8	862.2	1,105.6	1,118.8	990.0	708.6	1,132.8	926.8	838.2	805.4	976.0	983.6	1,050.4	781.4	906.2
6	1,108.2	1,034.2	820.8	975.8	967.0	1,017.4	680.2	956.4	1,047.2	1,104.8	989.8	1,215.4	962.2	1,001.8	918.0	976.6
7	1,049.4	971.0	1,104.8	1,223.8	981.4	955.2	781.0	1,088.0	1,131.6	995.6	880.8	1,090.2	935.8	992.8	988.2	1,077.8
8	1,124.4	1,112.0	1,018.8	1,341.8	1,178.4	1,053.4	1,013.2	1,422.6	1,342.6	1,026.8	1,010.4	1,088.4	982.6	1,150.6	1,003.2	1,231.8
9	1,065.8	960.0	961.6	1,161.2	829.0	889.4	794.0	987.4	962.0	1,033.0	1,097.4	1,139.4	1,067.8	962.6	824.8	914.2
10	840.6	914.0	886.4	993.0	1,237.2	1,031.0	1,021.0	1,092.6	969.0	999.6	1,127.0	1,251.2	977.2	993.2	1,103.6	1,181.8
11	953.8	1,104.4	891.8	1,311.2	1,124.6	1,029.4	854.0	1,326.2	1,049.2	888.0	885.8	1,101.2	981.2	1,209.0	1,011.6	1,277.0
12	1,004.2	1,251.8	1,142.2	1,541.0	1,159.4	1,245.6	1,070.8	1,331.6	982.6	1,306.8	1,018.2	1,321.4	1,388.0	1,230.2	1,028.0	1,326.8
13	1,259.6	1,161.2	1,188.4	1,198.6	1,099.4	1,155.2	1,035.0	1,282.0	1,076.0	1,026.4	1,138.0	1,226.0	1,226.4	1,401.0	1,016.8	1,260.8
14	841.6	1,019.4	869.0	1,150.4	984.6	1,050.0	981.8	1,094.4	963.8	1,135.6	959.4	1,236.8	1,152.4	1,036.4	905.4	1,039.0
15	1,491.0	1,275.4	1,142.4	1,588.6	1,210.6	1,419.0	1,210.8	1,512.6	1,298.8	1,170.8	1,437.0	1,492.2	1,391.4	1,284.0	1,138.0	1,343.0
16	951.0	877.8	933.0	1,065.6	854.6	884.0	889.0	905.0	977.4	1,009.2	749.2	1,082.2	949.6	971.2	803.2	1,220.4
17	1,008.2	867.4	944.2	972.8	728.2	761.4	594.8	891.4	1,014.8	1,026.2	979.8	1,093.2	980.8	831.8	1,011.4	1,043.6
18	1,122.2	1,127.8	999.4	1,240.4	1,201.2	1,197.2	1,239.8	1,082.6	1,217.6	1,204.2	1,031.6	1,393.8	1,246.6	1,285.2	1,186.2	1,261.8
19	1,254.2	1,112.2	1,128.0	1,404.6	985.6	1,045.8	963.0	1,010.8	977.8	865.8	1,011.0	995.0	1,074.2	986.6	947.6	1,121.4
20	915.8	980.2	908.4	1,158.0	963.6	900.2	768.4	862.2	761.0	965.8	961.0	1,029.0	829.0	1,000.6	1,013.2	887.0
21	1,184.2	1,061.0	925.8	1,120.6	1,005.8	980.6	829.6	1,151.6	1,092.2	1,165.2	943.2	1,086.4	1,086.6	1,031.8	1,051.2	937.6
22	1,069.4	1,017.0	939.2	1,261.4	1,140.6	1,083.8	1,122.2	1,121.4	1,140.0	1,214.6	973.6	1,176.8	1,014.6	994.2	1,270.8	1,088.2
23	919.6	1,022.2	944.6	1,054.8	1,030.6	965.6	852.8	982.4	1,047.8	1,042.8	876.0	1,112.4	1,048.2	982.4	939.0	1,040.6
24	1,043.8	1,188.2	855.2	1,161.2	1,115.2	1,187.0	814.4	1,087.2	1,301.0	1,245.6	915.6	1,140.2	1,149.8	1,101.2	1,094.6	1,138.0
25	891.8	954.0	995.6	924.2	938.6	1,002.6	797.8	955.4	961.0	1,020.2	788.2	908.0	962.2	1,043.6	837.6	1,040.8
26	1,121.2	1,185.6	941.6	1,273.6	1,045.8	909.2	910.6	1,059.2	1,056.4	1,011.2	915.8	946.7	1,149.0	1,128.8	959.0	983.6
27	1,135.8	1,144.6	1,003.8	1,112.4	1,046.0	1,145.0	1,017.8	1,259.2	1,086.0	1,130.4	1,280.2	1,294.0	1,188.8	1,146.6	884.6	1,129.8
28	765.6	921.4	858.0	1,004.2	1,088.8	945.4	934.2	881.8	1,031.6	1,077.6	939.8	1,096.8	928.2	907.0	948.0	1,042.8
29	865.2	821.8	731.8	919.6	892.2	954.0	924.2	705.8	819.4	900.8	1,106.0	950.0	773.4	811.6	812.2	978.8
30	959.6	1,043.0	1,029.6	969.7	1,095.4	1,000.6	1,030.0	1,081.2	994.8	970.8	887.4	1,105.2	920.6	894.6	825.8	1,003.6
31	1,178.2	1,121.0	1,155.6	1,285.2	1,128.2	1,109.0	994.2	1,153.0	955.6	940.6	1,055.4	1,160.0	938.8	1,097.8	972.8	1,041.4
32	1,021.8	1,131.2	923.4	1,080.4	1,062.2	906.4	967.2	998.6	882.0	849.6	804.8	934.4	1,070.8	1,041.2	925.8	1,175.4
33	1,164.8	1,100.2	880.6	1,116.0	1,124.6	1,072.4	983.8	1,054.0	1,086.8	1,106.6	920.6	973.2	1,047.2	1,285.6	1,118.8	1,142.2

*The value for Quadrate lobe-B4 in case number 4 is missing due to severe autolysis in the tissue.

Histopathological findings

Absence of severe diffuse hepatic diseases such as cirrhosis, massive necrosis, or primary neoplasm in all the examined specimens was histologically confirmed.

Measurement of the HLD

Measured values of the HLD are shown in Table 2. The HLD of the following samples could not be obtained due to severe autolysis or absence of measurable portal tracts or central veins in the given specimens: 5 of 5 Q-B4 in case 4; 3 of 5 Q-B4 in case 13; 2 of 5 RM-B4 in case 26; 1 of 5 RM-B4 and 1 of 5 RL-B4 in case 27; 3 of 5 RM-B2 in case 29; 2 of 5 LL-B4 in case 30. This resulted in one missing value for Q-B4 in case 4. Average of all the other measured values was 1.042 mm.

Statistical analysis

Analysis 1: Is there a significant difference among the HLD values of each sampling location (E, B, B2, B4) in a given hepatic lobe?

A Shapiro-Wilk normality test for all the data showed a normal distribution with the exception of the data for RM-B2. The RM-B2 values were further evaluated by drawing a normal Q-Q plot to visualize the degree of deviation (data not shown), then judged to be sufficiently normally distributed below the value of 1.200 mm of the horizontal axis. These results warranted the application of a parametric test for all the examined values. Plots of the mean and its 95% confidence interval (95%CI) of the HLD values of each sampling location in LL, Q, RM, RL are shown in Fig. 3 to visualize the distribution of the measured values. *P*-values of the repeated measures ANOVA and subsequent Bonferroni multiple comparison test for samples of all the 4 lobes and all 4 sampling locations are shown in Table 3.

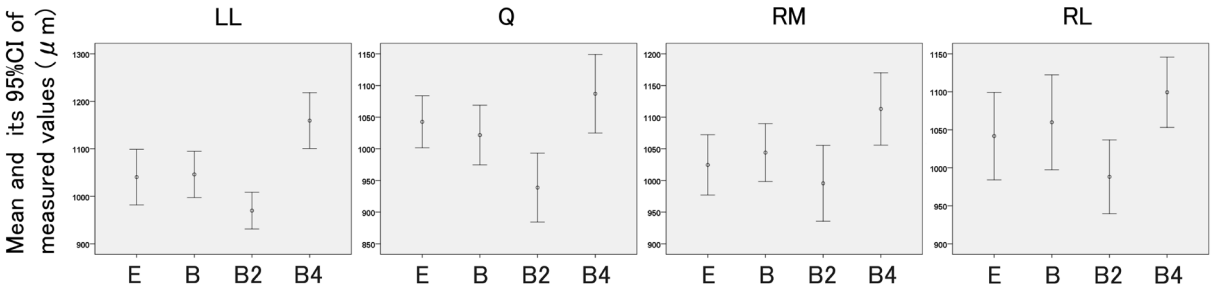

Fig. 3. Plots of the mean and its 95% confidence interval of the hepatic lobular diameter values of each sampling location in left lateral, quadrate, right medial, and right lateral lobes.

Table 3. Results of Bonferroni multiple comparison test for samples of all 4 lobes and all 4 sampling locations

	Overall	E vs B	E vs B2	E vs B4	B vs B2	B vs B4	B2 vs B4
LL	0.000[a)]	1.000	0.022[a)]	0.000[a)]	0.004[a)]	0.000[a)]	0.000[a)]
Q	0.000[a)]	1.000	0.000[a)]	0.516	0.001[a)]	0.054	0.000[a)]
RM	0.000[a)]	1.000	1.000	0.009[a)]	0.568	0.016[a)]	0.000[a)]
RL	0.000[a)]	1.000	0.316	0.103	0.048[a)]	0.531	0.000[a)]

a) $P<0.05$.

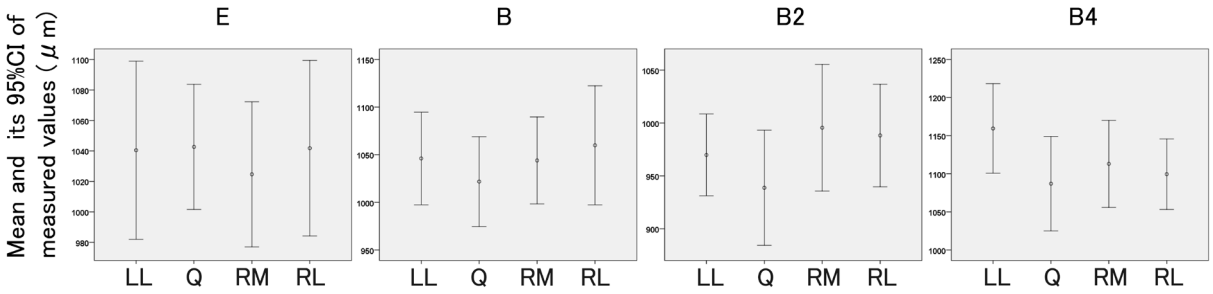

Fig. 4. Plots of the mean and its 95% confidence interval of the HLD values of each hepatic lobe at E, B, B2, and B4.

In LL and Q, B2 showed the smallest average measured values and B2 showed a statistically significant difference against any other sampling locations.

In RM, B2 showed the smallest average measured values; however, B2 only showed a statistically significant difference against B4.

In RL, B2 showed the smallest average measured values; however, B2 only showed a statistically significant difference against B and B4.

Analysis 2: Is there significant difference among the HLD values of each lobe (LL, Q, RM, RL) at a given sampling location?

A similar statistical approach taken for Analysis 1 was conducted. Plots of the mean and its 95%CI of the HLD values of each hepatic lobe at E, B, B2, B4 are shown in Fig. 4.

Regardless of the sampling location (B, B2, and B4 except E), the average measurement value of Q tended to be smaller than that of the other lobes; however, a statistically significant difference was present only between LL and Q of B4 (P=0.030).

HLD measurement in biopsied liver samples from PPVH dogs

Signalment, clinical information, abnormal blood test values, and the average HLD values are summarized in Table 4. None of these dogs had ascites or secondary extrahepatic shunt vessels. The results of complete blood counts were within the reference range. All biopsy specimens were taken from the edge of the left lateral lobe. Figure 5 shows multiple hepatic lobules with markedly reduced HLD in the liver of case 35.

DISCUSSION

From this study, the average of all the measured values of HLD was 1.042 mm, which corresponds to the lower limit of an anecdotal range of the HLD found in the veterinary literature [4]. To our knowledge, this is the first report of an established value for the average HLD derived from a steady measurement of multiple liver samples of client-owned dogs of various, but mainly

Table 4. Summary of signalment, clinical information, blood test results, and the average hepatic lobular diameter (HLD) values of the dogs with primary portal vein hypoplasia (PPVH)

Case number	Age	Sex[a)]	Breed	Clinical information[b)]	Blood test results[c)]	HLD[d)]
34	12y5m	SF	Miniature Dachshund	No clinical sign; microhepatia (X-ray); gallbladder sludge (US)	ALT 146, AST 155, ALP 429, TBIL 0.9, 2-hr-postprandial TBA 30.5	579
35	10y4m	SF	Yorkshire Terrier	Intermittent vomiting for 1 month; gallbladder sludge and small stones (US); microhepatia (intraoperative observation)	ALT 262, AST 35, ALP 136, TBIL 0.3	675
36	10y7m	CM	Chihuahua	No clinical sign; microhepatia (X-ray); gallbladder sludge and small stones (US)	ALT 161, AST 87, ALP 116, TBIL 0.1	643
37	10y9m	SF	Pug	No clinical sign; microhepatia (X-ray); gallbladder immovable contents (US)	ALT 81, AST 83, ALP 91, TBIL 0.3, 2-hr-postprandial ammonium 122, 2-hr-postprandial TBA 38	594
38	7y3m	CM	French Bulldog	No clinical sign; microhepatia (X-ray); gallbladder sludge (US)	ALT 100, AST 30, ALP 66, TBIL 0.1	550

a) SF=spayed female, CM=castrated male. b) US=ultrasound. c) Reference interval: ALT (alanine transaminase) 17–78 U/*l*; AST (aspartate transaminase) 17–44 U/*l*; ALP (alkaline phosphatase) 47–254 U/*l*; TBIL (total bilirubin) 0.1–0.5 mg/d*l*; 2-hr-postprandial ammonium <50 μg/d*l*; 2-hr-postprandial TBA (total bile acid) <25 μmol/*l*. d) Average of five (case 1–4) or four (case 5) hepatic lobules.

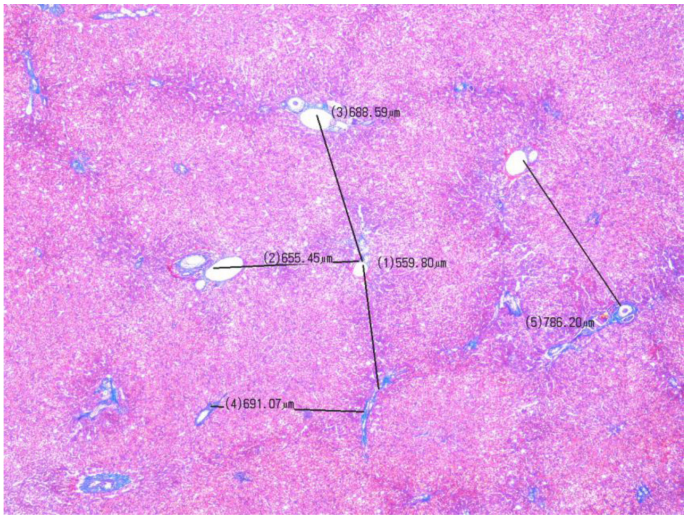

Fig. 5. Photomicrograph showing measurement of the hepatic lobular diameter using a histospecimen of the canine liver (case 35, left lateral lobe). Masson trichrome stain. Note the smaller size of hepatic lobules compared to hepatic lobules in Fig. 2.

smaller breeds. This result, however, should be carefully extrapolated to the diagnostic or research settings. First to consider will be the lack of knowledge on the consistency of the HLD values among numerous canine breeds, whose body size tremendously varies from a 3-kg Miniature Poodle to a 60-kg Great Dane. Though we tend to presume that there is not such a big difference among the HLD values of these contrasting canine breeds from mere experience of routine examination of canine liver histospecimens, there is definitely a need for further research in which breed differences are carefully taken into consideration. The second challenge is related to methodology, especially to the effect of a possible shrinkage artifact during histology tissue preparation [3]. Because most liver biopsy samples should go through processes of fixation, paraffin embedding, and sectioning, the value we obtained (1.042 mm) is most likely smaller than that of the "actual" one. However, since measurement of the HLD is always performed in routinely prepared histospecimens, the second concern regarding shrinkage artifact may not interfere with our daily diagnostic pathological work including measurement of the HLD. The third concern is related to the fact that the examined livers were not from those dogs whose status had been objectively judged "good" by standardized clinical diagnostics. Rather, some dogs in this study suffered from severe, if not diffuse, hepatic pathology. Therefore, in order to establish reference range for HLD values of healthy dogs, measurement should be performed for a large cohort of dogs with guaranteed health status.

The HLD tended to be the smallest in samples taken from location B2 regardless of topology (i.e. liver lobes) from which the samples had been taken. In addition, the smallness of the HLD in B2 specimens was judged statistically significant when compared to the samples from other locations (E, B, B4) in two liver lobes (LL, Q). In the remaining lobes (RM, RL), statistical significance of smallness of the HLD in B2 was shown between B2 and B4 (RM) and between B2 and B as well as B2 and B4 (RL), respectively. These results indicate that at a depth of 2 mm below the hepatic capsule, the HLD is smaller than in other

areas. It is possible that the subcapsular area of the liver is less perfused by the tapering portal veins and hepatic arteries toward the capsule, thus this subcapsular area may be undernourished resulting in smaller hepatic lobules than in the deeper parenchyma. The possibility that the HLD in the subcapsular 2-mm area is normally smaller than that in the other areas is important for veterinary practitioners, histotechnicians, veterinary pathologists, and researchers to know in order to avoid overinterpretation of the smallness of the HLD in this area. The reason why emphasizing this is because some veterinary practitioners prefer to take liver biopsy samples through "scooping/spooning" method, with resultant biopsy tissue being very thin, usually less than 2 mm. Otherwise we can be led to a misdiagnosis of canine PPVH for patients that are actually free of this disease. Of interest, in humans, histological examination of a subcapsular 2-to-10-mm-thick area of the liver needs precaution because misinterpretation as hepatic cirrhosis can occur due to a natural abundance of fibrous tissue in this area [14, 23]. The reason for this abundance of fibrous tissue in the subcapsular area in human livers, however, is unknown. In order to adequately evaluate the liver biopsy specimen, avoidance of examination of the subcapsular 2-mm area seems to be the key in both human and veterinary medicine. Thus, both human and animal pathologists should instruct their colleague practitioners and histotechnicians not to collect or trim the shallow subcapsular hepatic parenchyma for histological evaluation. In case it is inevitable to do so, pathologists then have to be cautious taking measurements of the HLD in that particular area. Also, one should not use a scalpel parallel to the hepatic capsule in the shallow (1–2 mm deep from the capsule) parenchyma in trimming of the biopsied liver specimen, but instead, should make an incision vertical to the capsule in order to include the deepest portion of the sample for microscopic evaluation. This guideline should be shared among pathology laboratory personnel in order to ensure a proper diagnosis.

As for the importance of the choice of liver lobes (LL, Q, RM, RL) on the measurement of HLD, regardless of the sampling location, the average measurement value of Q tended to be smaller than that of other lobes; however, a statistically significant difference was present only between LL and Q of B4. These results would suggest that the choice of hepatic lobe for biopsy can be arbitrary and taking a sample from a single lobe likely results in representative data on the general status of the liver mass of the patient unless there is a particular interest or purpose of the examiner in pursuing that biopsy. The risk of general anesthesia or biopsy-related complications can be minimized by reducing the number of samples i.e. reducing total anesthesia time and attempts of surgical intervention as we hereby demonstrated. Also, it would be deemed advisable that one avoid taking a single sample from the quadrate lobe because the HLD values of this particular lobe may measure smaller than in specimens from the other lobes.

The relationship among the measured HLD values, breed, and sex of the dogs was beyond the scope of the present study. Further investigation using multiple (statistically adequate numbers) subjects/samples from an adequately randomized animal pool is necessary to address this issue. The HLD tends to be smaller in small-breed dogs than in large breeds (unpublished data). Since smaller dog breeds are popular in Japan likely due to housing constraint, liver samples or measured HLD values of the dogs of a larger breed may need to be obtained from collaborators in other nations.

PPVH is essentially a veterinary disease while other congenital vascular disorders affecting circulation of the liver, such as PSS, have been reported with precise histologic description in human medicine [10]. Discussing the existence (underdiagnosis) of PPVH in people is beyond the scope of our study; however, the authors speculate that idiopathic non-cirrhotic intrahepatic portal hypertension of humans could be a potential counterpart of PPVH of animals due to their common gross and histological findings as well as the relatively benign clinical course in both diseases [1, 5, 21, 25]. The reason PPVH is often diagnosed in dogs in their adolescence seems to be related to a regular (mostly annual) health check in canine medicine. In fact, these days, many client-owned dogs in developed societies have medical checkups from the age of 1 year or less (corresponds to adolescence in people) and it continues toward their senility. The health check of a dog usually covers a variety of items, i.e. complete blood count, blood chemistry, and a test for infestation status of *Dirofilaria immitis*. In addition, many veterinary hospitals offer an extra set of examinations such as US, radiology, and/or endocrinology tests on dogs so that subclinical or covert abnormalities like PPVH can be found efficiently. There is another advantage for the early detection of PPVH in dogs in that laparotomy or laparoscopy is a popular approach for the diagnosis, manipulation, and/or treatment of various intra-abdominal conditions of dogs. Ovariohysterectomy is also generally recommended for female dogs to prevent sex-hormone associated disorders such as the development of mammary neoplasm in later life [15]. Therefore, the resistance against performing surgical biopsy of the liver in animals, especially in dogs, may be much lower than in human medicine. A large repository of liver samples of sufficient size is needed to aid in much needed research on canine hepatopathology. Spontaneous liver diseases of dogs can also provide potential models of human disorders. For example, chronic hepatitis of Bedlington Terriers is considered to be a counterpart of human Wilson disease (copper storage disorder) [8, 11].

Though currently not required for histological diagnosis of PPVH, an accurate measurement for the standard HLD of a normal hepatic lobule is essential to the pathologist in order to determine whether smaller hepatic lobules are normal or signify signs of disease. In the present study, average HLD values of the five PPVH dogs (579 μm, 675 μm, 643 μm, 594 μm, and 550 μm, respectively) were smaller than the tentative standard value of HLD (1,042 μm). These results indicate the potential importance of measuring HLD as an objective assessment of hepatic disorders of unproven pathogenesis. The HLD, as it likely represents a collection of numerous hepatocytes, can be a handy surrogate for the measurement of individual hepatocytes, whose diameter can fluctuate significantly due to various factors such as metabolism, blood supply, degeneration, regeneration, neoplastic proliferation, fixation, tissue handling, and intra- or inter-observer dissimilarity at microscopic evaluation. In addition, the liver of dogs has anatomical similarity to the human liver: sinusoids of humans and dogs reportedly drain solely into the central veins whereas they enter hepatic veins at all levels in rodents [12]. Investigation of the dog liver thus may accelerate understanding of the human liver pathophysiology more efficiently than using rodent models. Finally, the authors propose that pathologists, regardless of expertise, take the HLD values in their routine liver assessment in order to more accurately record the changes in this multi-task intriguing organ.

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