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Retrospective liver histomorphological analysis in dogs in instances of clinical suspicion of congenital portosystemic shunt

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Abstract

Introduction: The clinical symptoms of portosystemic shunts (PSSs) and hepatic microvascular dysplasia (HMD) – portal vein hypoplasia (PVH) in dogs are similar. PSSs are abnormal vascular connections between the portal vein system and systemic veins. HMD is a very rare developmental vascular anomaly, recognisable during histopathological examination. The study aim was to assess the prevalence of HMD–PVH and hepatocellular and vascular pathologies in the liver. **Material and Methods:** Liver biopsies from 140 dogs (of different breeds and both sexes) arousing clinical suspicion of PSS were examined histopathologically. **Results:** An initial PSS diagnosis was confirmed in 125 dogs (89.29%). HMD–PVH was found in 12.32% of dogs, as an isolated disease in 9.29%, especially in Yorkshire terriers, and with extrahepatic PSS in 6.67%. Histopathological analysis of muscles around sublobular veins showed that HMD cases presented hypertrophy or hypertrophy with fibrosis. In 2.17% of all dogs with liver vascular developmental disorders calcification was visible around vessels (without correlation by degenerative changes in those vessels), suggesting prior onset of deep metabolic disorders. Clinical suspicion of PSS was also formed upon quite different pathological processes in young dogs. **Conclusion:** Histopathological findings diagnosed the type of vascular anomalies (PSS or HMD–PVH) or other pathological changes conclusively, therefore detailed hepatic histopathology is an indispensable component of the clinical diagnostic process.

Keywords: dog, hepatic microvascular dysplasia, congenital portosystemic shunt, histopathology, liver.

Introduction

Portosystemic shunts are abnormal vascular connections between the portal vein system and systemic vein circulation which allow the blood to flow directly into the circulation system, bypassing the liver (9, 17). Shunts are either congenital or acquired. The more commonly encountered, congenital portosystemic shunts (PSSs), constitute pathological vessels that developed during the embryonic stage; in general, they are single (both extra- and intrahepatic shunts) and their presence is not associated with portal hypertension. Different pathologies of the portal vessel system have been reported in the dog (3).

Single congenital intrahepatic shunts connect the portal vein with the caudal vena cava. Left-sided instances (left divisional shunts) originate from the abnormally closed foetal *ductus venosus*. The pathogenesis of intrahepatic PSSs in right medial and right lateral liver lobes is unknown (5, 9, 17). Intrahepatic shunts constitute approximately a quarter of reported cases in the dog (11).

Extrahepatic shunts usually connect the portal vein or one of its affluxes (left gastric vein or splenic vein) with the caudal vena cava cranially to the phrenicoabdominal veins (5, 9). Less commonly, an abnormal vessel connects with the azygous vein. Single extrahepatic shunts result from abnormalities that occur during the development of circulation in the vitelline system (10).

Congenital shunts are more often reported in pedigree dogs than in cross-breeds (9). Intrahepatic shunts are usually diagnosed in large breeds (Dobermann pinschers, golden retrievers, Labrador retrievers, Irish setters, Samoyeds, or Irish wolfhounds), whereas extrahepatic shunts are reported in small-breed dogs (such as Yorkshire terriers, miniature schnauzers, Cairn terriers, Maltese, miniature poodles, or dachshunds) (11).

The clinical symptoms of congenital portosystemic shunt are usually noted in young dogs, aged <1 year (3) with the average age ranging from 2 months to 10 years. Sex predilection is not reported. The symptoms are extremely varied and depend on the size and location of the shunt (11). Hepatic encephalopathy predominates and it is caused by insufficient elimination of toxic compounds originating from blood in the gastrointestinal tract (ammonia, mercaptans, short-chained fatty acids, γ-aminobutyric acid, and endogenous benzodiazepines (9). These symptoms are generally mild (anorexia, depression and lethargy), although they may be severe and present as ataxia, seizures, dizziness, and coma. These signs worsen after ingestion of a high-protein meal and may change over a day or a week (9, 11). In addition, non-specific gastrointestinal symptoms are reported (diarrhoea, vomiting, inappetance and polydipsia) and these signs in puppies are accompanied by retarded growth and lower body weight or even emaciation. Furthermore, urinary symptoms are sometimes observed such as polyuria, urolithiasis (a complication in 50% of animals with congenital PSS) and haematuria (9). Laboratory tests most often reveal hypoalbuminaemia, hypoglobulinaemia, hypoglycaemia, a mild increase in AP, ALT and AST activities, hypocholesterolaemia, and elevated pre-prandial and high post-prandial concentration of bile acids (3, 9). Moreover, mild non-regenerative microcytic anaemia is reported (11). Liver atrophy may also be observed (5, 10).

The histopathological pattern reveals a lack of portal veins and an increased number of arterioles (that are often tortuous) in the hepatic triads as well as atrophy of hepatocytes with lipogranuloma formation, and sometimes sinusoidal dilatation around the portal areas (5). Hepatic veins may have prominent smooth muscles (17). The size and location of the shunt is the determinant of the severity of the lesions which are typical for portal vein hypoperfusion (5).

Similar clinical symptoms are reported in dogs with hepatic microvascular dysplasia (HMD), which is an extremely rare developmental pathology of blood vessels observed in the histopathology of the liver (4, 8). Some researchers categorise it as primary hypoplasia of the portal vein (PVH) (5, 11). Disseminated small or juvenile-like vessels, central venous mural hypertrophy with smooth muscle proliferation with or without fibrosis, dilatation of periportal vascular areas, an increased number of arterioles in the hepatic triads and hyperplasia of the endothelium in the portal areas are observed in the liver (4, 17). Some authors have also reported hyperplasia of hepatic stellate cells (Ito cells) and Browicz-Kupffer cells as well as a decrease in the diameter of intrahepatic portal veins (4). In about 30% of dogs with primary portal vein hypoplasia the vascular changes are mild and there is no portal fibrosis (5). On the other hand, portal vein hypoplasia (formerly termed hepatic microvascular dysplasia) can occur as an isolated disease or in conjunction with macroscopic portosystemic shunts (3, 11).

Hepatic microvascular dysplasia most often affects Yorkshire terriers and Cairn terriers, however it is also diagnosed in Maltese, dachshunds, poodles, shih tzu, Lhasa apsos, cocker spaniels and West Highland white terriers (7, 9). In Cairn terriers, this disease has a genetic background (11) and may be inherited polygenically (7). In these dogs it may present without noticeable clinical symptoms (9).

The objective of the paper was to assess hepatocellular and vascular pathologies in the microscopic pattern of the liver in dogs with the clinical symptoms of portosystemic shunts as well as to evaluate the prevalence of portal vein hypoplasia, based on the histopathological examination of surgically collected biopsy specimens of the liver.

Material and Methods

The study was carried out on archival paraffin blocks collected in 2005–2014, which included 140 surgically biopsied liver samples from dogs (of different breeds and both sexes, which aroused clinical suspicion of congenital portosystemic shunt) during diagnostic laparotomies or surgical closures of pathological vascular shunts. The physical examination of all dogs revealed evident and severe symptoms: non-specific gastrointestinal and neurological signs suggestive of portosystemic shunts, and the blood tests showed elevated levels of AP, ALT, AST, ammonia, and bile acids. Bile acid levels were: 24–113 micromoles/L pre-prandial, 68–212 micromoles/L post-prandial (reference: up to 20 micromoles/L pre-prandial, up to 25 micromoles post-prandial (3)). The ammonia levels was 43–278 micromoles/L (reference: up to 70 micromoles/L). In 139 animals shrinkage of the liver was observed.

A male and a female Yorkshire terrier aged 11 months and 5 years, respectively, had been previously diagnosed and the male was treated for urolithiasis for four months. Apart from neurological symptoms, haematuria was found together with deposits in the

urinary bladder (evidenced by ultrasound or X-ray examination) and microcytic anaemia detected in blood tests. During clinical examination of the female some more abnormalities were found: os penis in the vulva and abnormal left gonad adherent to the urinary bladder.

The specimens from the liver were sampled by the "guillotine" method (one sample from each animal): a Roeder loop of absorbable suture (Safil 1-0; B. Braun, Germany) was placed around the protruding margin of a left lateral lobe or a papillary process of the caudate lobe. The loop was pulled tight, and it was allowed to cut through the hepatic parenchyma before it was tied. After tying the ligature the hepatic tissue was cut approximately 3–5 mm distal to it.

The samples for histopathological examination were routinely fixed in buffered 10% formalin, then embedded in paraffin (Paraplast), cut, and stained with haematoxylin and eosin (HE). Masson's stain for collagen fibres, Perls' for iron, and the Köss method for calcium salts were also utilised (1, 2). In 10 cases immunohistochemical staining was performed with Polyclonal Rabbit Anti-Human Von Willebrand Factor (A 0082, DAKO Cytomation, Denmark). Samples of the liver from healthy young dogs (two years of age; killed in road accidents) and samples from neoplastic tissue with angiogenesis were used as controls.

Liver fibrosis determination was based on an adapted Scheuer staging scale (6, 18).

Results

The diagnostic laparotomy confirmed an initial diagnosis of congenital portosystemic shunt in 125 dogs (89.29%): extrahepatic portosystemic single shunt (EPSS) in 119 individuals (85.00%), extrahepatic multiple shunts in 1 animal (0.71%), and intrahepatic shunt (IPSS) in 5 patients (3.57%). Laparotomy did not confirm the presence of portosystemic shunt in 15 cases (10.71%). In 13 dogs (9.29%) the final diagnosis was hepatic microvascular dysplasia–portal vein hypoplasia (HMD–PVH). In one six-month-old male golden retriever ascending cholangiohepatitis and congenital haemangioma were diagnosed; idiopathic hepatic fibrosis was diagnosed in a female German shepherd aged five months. The diagnosis of extrahepatic portosystemic shunt, urolithiasis and hermaphroditism was made for one dog.

Yorkshire terriers (or dogs of this type of breed) dominated the group of dogs with EPSSs (51 out of 119 cases; 42.86%) and the group with HMD–PVH (9 out of 13 cases; 69.23%); the total number of such dogs in our study was 60 (42.86 % of the study population).

During clinical data analysis concerning Yorkshire terriers, it should be noted that among dogs with an extrahepatic portosystemic shunt, there were animals from three months to three years of age and a single five- or six-year-old. Initial diagnosis of a portosystemic shunt was made predominantly in animals between the ages of 6–9 months (13 dogs), another peak was recorded in 12–13 month-old dogs (9 dogs), and also in dogs aged 18–24 months (12 dogs). The age of dogs with portal vein hypoplasia ranged from four months to four years, with the largest number of animals diagnosed for the first time between their 4^{th} and 7^{th} months (4 dogs), and the next largest first ascribed PVH between their 11^{th} and 12^{th} months (3 dogs). Data on gender suggest that in Yorkshire terriers with EPSSs the number of males and females is comparable, while males predominate in the group of dogs with HMD–PVH (60%).

In the group of dogs with intrahepatic shunts, 60% were cross-breeds and 40% German shepherds. Urolithiasis was diagnosed in two dogs (1.45% of all dogs with vascular developmental disorders and 1.60% of dogs with EPSSs).

Histopathological lesions are presented in Figs 1 and 2, and in Tables 1–3. In all investigated groups the histopathological examination showed similar changes: significantly decreased vein diameter or absent veins in the hepatic triads; an increased number of arterioles (in dogs with EPSSs predominantly 2 arteries as seen in 51 dogs, or 3 arteries as seen in 45 dogs; in dogs with IPSS predominantly 3 arteries as seen in 3 dogs; in dogs with HMD–PVH predominantly 2 arteries as seen in 7 dogs); and randomly disseminated small blood vessels in the liver parenchyma, which were tortuous in some dogs and accompanied by fibrosis of the periportal areas. In five dogs with EPSSs (4.17%) changes in the diameter of veins in the portal areas were minimal. In two dogs with EPSSs, dilatation of the sinusoids around the portal area resembling phlebectatic peliosis was found. Changes in the smooth muscles around sublobular veins were noted as follows. In EPSS diagnoses there was hypertrophy in nine cases, fibrosis in nine cases, and both in ten cases; where IPSS was confirmed hypertrophy was observed in one dog; and in HMD–PVD–afflicted animals hypertrophy came to light in three cases, fibrosis in five cases, and both in three cases, (Fig. 2B). Furthermore, hyperplasia of the endothelium of the central vein was reported; in 15 cases of EPSS it was seen in five dogs with fibrosis; in four dogs with IPSS it was present in two animals with fibrosis; and it was manifested in four patients diagnosed with HMD–PVH (Fig. 2A). In another 12 dogs with EPSSs, fibrosis around the central vein was visible.

In 23 cases of EPSS (19.17%) where the liver parenchyma was completely rebuilt, it was difficult to determine its structure and define the portal area or types and courses of vessels. Immunohistochemical staining showed that in such cases, especially with lipogranuloma formation, endothelial cells of vessels are present between macrophages and hepatocytes, but the course of vessels is significantly different from that of vessels in the healthy liver (Figs 1C and D).

In eight dogs (6.67%) with confirmed EPSSs, changes in the smooth muscles around sublobular veins and lesions in the central vein walls were noted together, so these dogs also showed hepatic microvascular

dysplasia. Two of them (Yorkshire terriers which were a female aged 10 months and a male aged 11 months) died shortly after the procedures of surgical closures of pathological vascular shunts (10 and 2 days later, respectively).

Dilatation of lymphatic vessels was reported in 62 cases of EPSS, 3 cases of IPSS, and 3 cases of HMD–PVH. It was predominantly correlated with the presence of inflammatory cells (scant mononuclear cell infiltrate) around the portal area.

Lesions were present in hepatocytes which varied in severity between patients and included necrosis, focal or diffused small-droplet or large-droplet fatty degeneration, atrophy, and parenchymatous degeneration. Lipogranulomas or pigment granulomas of different sizes were found. In 2.17% of all dogs with liver vascular developmental disorders (in two cases of EPSS: a male miniature schnauzer of 12 months of age, and a male cross-breed, of 8 months of age; and in one case of HMD-PVH: a male Yorkshire terrier of 4 months of age) calcification around the vessels or calcification of the vessel walls was noted (Figs 1A and B).

Steroid-induced hepatopathy associated with the drugs administered before the procedure was observed in some individuals (42 dogs) of all groups.

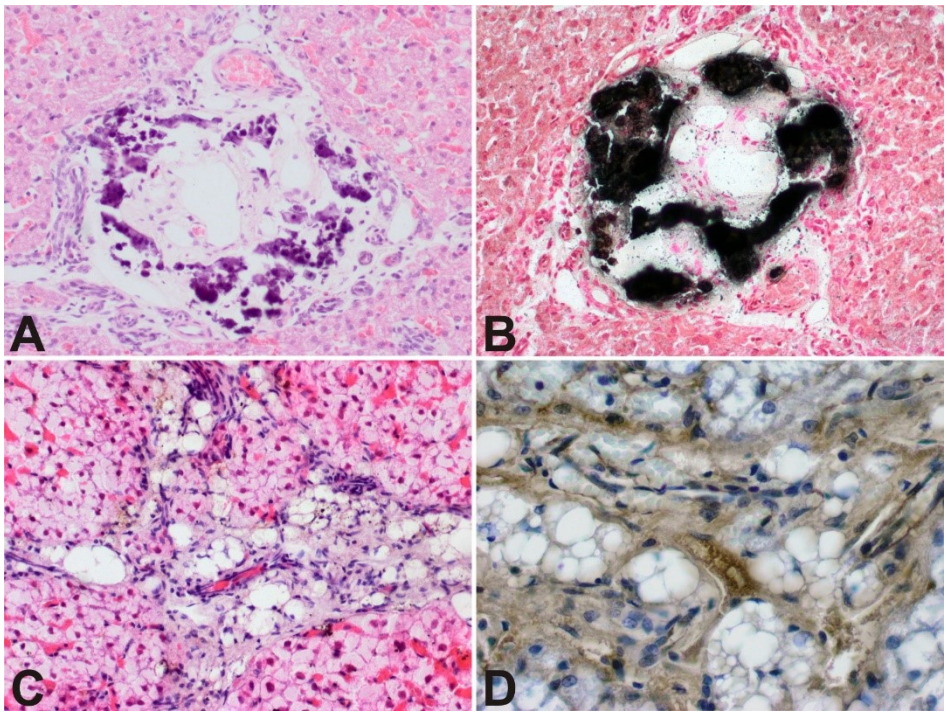

Fig. 1. Microscopic pattern of the liver in dogs with EPSSs. A and B – calcium salts deposits around the vessels (A – purple, HE, B – black, Köss stain, 20×); C – vascular abnormalities, steroid-induced hepatopathy and lipogranuloma formation (HE, 10×); D – immunohistochemical demonstration of endothelial cells (brown staining for von Willebrand Factor, 40×)

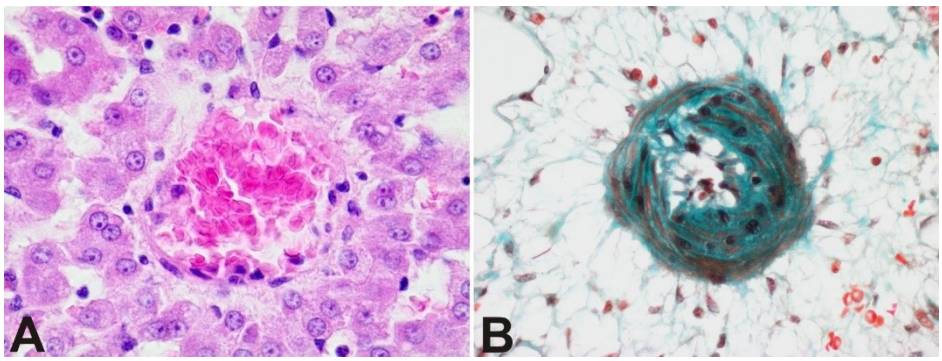

Fig. 2. Microscopic pattern of the liver in dogs with HMD–PVH. A – hypertrophy of the endothelium of the central vein wall (HE, 40×); B – hypertrophy and fibrosis (green) of the smooth muscles around sublobular veins (Masson stain, 40×)

Table 1. Histopathological lesions in liver parenchymatous cells (hepatocytes)

Type of histopathological lesions	Extrahepatic portosystemic shunt – % of cases		Intrahepatic portosystemic shunt – % of cases	Hepatic microvascular dysplasia-portal vein hypoplasia – % of cases
necrosis	disseminated	82.50	80	84.62
	focal	3.33	-	-
fatty degeneration		70.00	80	76.92
atrophy		60.83	80	69.23
parenchymatous degeneration		35.83	40	30.77
lipo/pigment granulomas		54.17	20	15.38

Table 2. Histopathological lesions in the vascular system of the liver

Type of histopathological lesions	Extrahepatic portosystemic shunt – % of cases	Intrahepatic portosystemic shunt – % of cases	Hepatic microvascular dysplasia–portal vein hypoplasia – % of cases
significantly decreased diameter of veins or absent veins in the portal triad	39.17	40	30.77
lesions in the central vein	22.50	60	23.08
lesions in the smooth muscles around sublobular vein	23.33	20	84.66
arteriole profiles in the portal areas	2–6 predominantly 2–3	2–6 predominantly 3	1–3 predominantly 2

Table 3. Liver fibrosis – adapted Scheuer scale

Stage of fibrosis	Extrahepatic portosystemic shunt	Intrahepatic portosystemic shunt	Hepatic microvascular dysplasia–portal vein hypoplasia
0/no fibrosis	13	1	0
0–1	17	0	2
1	23	1	1
1–2	23	2	3
2	16	0	6
2–3	16	0	1
3	6	1	0
3–4	5	0	0
4	1	0	0
liver fibrosis (total number of cases/%)	107/89.92	4/80	13/100
collagen fibres in the sinuses (total number of cases/%)	33/27.73	3/60	8/61.54

Discussion

Our findings are largely consistent with the data reported in the literature. Congenital extrahepatic shunts were diagnosed mainly in small pedigree dogs, whereas intrahepatic portosystemic shunts were diagnosed in large-breed dogs and their crosses. In our study, only 4% of examined dogs with clinically confirmed shunts were cases of the intrahepatic variety. According to Richter (11), intrahepatic shunts constitute approximately a quarter of reported cases in the dog. Such a large difference can be explained by the fact that the material for the research was acquired in a metropolitan clinic. In recent times, there has been an increasing interest in keeping small breeds in the city. Among small-breed dogs Yorkshire terriers were predominant and were within the age range corresponding to the data in the literature (3, 11). As previously stated, Yorkshire terriers (or dogs of this type of breed) are very popular as companion dogs in Poland, which may explain the dominance of this breed of dog among our patients (15).

In a subgroup of the examined animals, congenital extrahepatic shunts and hepatic microvascular dysplasia –portal vein hypoplasia were simultaneously diagnosed, which is also consistent with the reports in the literature (3, 4, 9, 11). In comparison with the dogs affected only by the shunts, these animals were more vulnerable to the effects of general anaesthesia during the procedure of closing pathological vascular connections, which is why two individuals died shortly after the surgery. The histopathological investigation of the liver helped us to determine the causes of the unfavourable outcome of the convalescence period.

Hepatic microvascular dysplasia without co-existing shunts was mainly diagnosed in Yorkshire terriers, which confirms that this breed is predisposed to this disease (7). In this group, the histopathological pattern of the liver was similar to that observed in the

dogs diagnosed with shunts alone. However, in the animals with dysplasia, hyperplasia of the arteries (seen in the portal areas) was less than in the dogs with shunts alone. Even if the cross-section of the vein was smaller in diameter than in the liver of the healthy dog, it was generally visible. In our previous investigations we determined that in animals with microvascular dysplasia the median diameter of the veins in the portal areas was 36.9 μm, whereas in dogs with congenital portosystemic shunts it was 19.1 μm (significantly smaller), and in healthy dogs was 82.1 μm (16). Moreover, fibrosis was more pronounced in dogs with portosystemic shunts compared to dogs with microvascular dysplasia. However, fibrosis of the liver was present in all dogs with microvascular dysplasia and in about 19% of animals with EPSSs, but the stage of fibrosis was more advanced in dogs with portosystemic shunts and could even present at stage 4.

Furthermore, lipogranulomas or pigment granulomas were found only in a few cases of microvascular dysplasia–portal vein hypoplasia. As proved by our previous analysis of liver samples from dogs with congenital EPSSs, formation of lipogranulomas is correlated not only with increasing age of the dog (and corresponding longer duration of the disease), but also with the diameter of vessels at the portal area and the diameter of pathological vessels circumventing the liver (15). In cases of HMD–PVH nutrient supply to the hepatocytes is limited because of the smaller diameter of veins than in the healthy dog; however, the diameter is larger than in a dog with a portosystemic shunt and there are no abnormal vessels circumventing the liver. In our opinion, this fact explains the less common occurrence of lipogranulomas in dogs with microvascular dysplasia than in dogs with portosystemic shunts.

In dogs, the sublobular veins are characterised by a wall with spiral shaped smooth muscles (5). Analysis of muscles around sublobular veins shows that in almost all cases of microvascular dysplasia there is hypertrophy or hypertrophy with fibrosis of these muscles. In our opinion, such pathology is common and even more important than hypertrophy of endothelial cells in the central vein or fibrosis around it.

The clinical symptoms and results of blood tests were similar in all dogs. A conclusive diagnosis was made based on the histopathological findings. The histomorphological evaluation of the organ led to an idiopathic hepatic fibrosis diagnosis in one German shepherd, in which a portosystemic shunt was ruled out during diagnostic laparotomy despite the clinical signs indicating its presence. Severe subcapsular fibrosis, absence of vascular pathologies (*i.e.* arteriole proliferation or decrease in vein diameter) in the portal area and absence of inflammatory cell infiltration determined the diagnosis of idiopathic hepatic fibrosis typical for German shepherds (14). The second case was a six-month-old male golden retriever with ascending cholangiohepatitis and congenital haemangioma (13). In this case hepatic histomorphological analysis gave an indication for antibiotic treatment to be started and that dog was subsequently restored to full health.

Urolithiasis was not a frequently coexisting pathology in our patients; it was observed in only two animals, which constituted 1.45% of all dogs with vascular developmental disorders and 1.6% of dogs with EPSSs. According to Johnson (9), it may be a complication in as many as 50 % of animals with congenital portosystemic shunts. In one dog with EPSSs and urolithiasis, hermaphroditism was also diagnosed (19).

During histopathological examination, calcification was visible around vessels in 2.17% of all dogs with liver vascular developmental disorders (two dogs with extrahepatic shunts and one with microvascular dysplasia). It did not correlate with degenerative lesions in vessel walls (dystrophic calcification). No information was found in the available literature about such pathological changes in the liver of dogs with portosystemic shunts or microvascular dysplasia. Animals with this kind of histopathological changes were young (4–12 months of age); moreover, calcification was a more frequent pathology than urolithiasis. This led us to conclude that in cases of congenital liver vascular anomalies, such as portosystemic shunts or microvascular dysplasia, more laboratory tests should be carried out in addition to determination of AP, ALT, AST, ammonia, and bile acids. In such animals, deep metabolic disorders may have manifested, and, if they had been correctly diagnosed earlier, they could have possibly been treated.

On the other hand, dogs with developmental anomalies of the liver vascular system are young animals, requiring a lifelong special diet and often costly surgical procedures, sometimes repeated several times (such as gradual closures of pathological vessels bypassing the liver). The prognosis in such cases is usually cautious and questionable. A detailed assessment of liver morphology and precise prognosis are important not only for health, but also for the animal's quality of life. They also have some economic importance and when owners are faced with high costs in cases of poor prognosis, euthanasia is indicated.

Immunohistochemical staining shows that in almost one fifth of cases, especially with lipogranuloma formation, precise diagnosis based only on routine (or additional histochemical) staining is impossible. In such cases immunohistochemical staining is an indispensable element of histopathological diagnosis. The conclusion drawn by the authors is that the application of routine, histochemical, and also immunohistochemical staining methods is used to objectify the result of a histopathological examination and helps to decide on further treatment (or withdrawal of treatment).

In summary, it is concluded that hepatic microvascular dysplasia is a frequent pathology in dogs with clinical symptoms of portosystemic shunts, especially in Yorkshire terriers. Histopathological

examination of the liver, following physical examination and diagnostic laparotomy, is an indispensable component of the diagnostic process and surgical biopsy is a good source of diagnostic material.

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Animal Rights Statement: The study was carried out with archival paraffin blocks, which included liver samples from dogs arousing clinical suspicion of congenital portosystemic shunt, obtained during diagnostic and therapeutic procedures.

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