Vacuolar hepatopathy in 43 French Scottish Terriers: a morphological study.

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SUMMARY
Scottish Terriers (STs) are known to develop vacuolar hepatopathy (VH) associated with elevated alkaline phosphatase (ALP) activity, which likely reflects a genetic defect affecting steroidogenesis that might also explain the appearance of hepatocellular carcinoma (HCCA) in these dogs. The objective of the present study was to assess the hepatic lesions in a cohort of French STs with VH using histological techniques and transmission electron microscopy (TEM) in order to characterize cellular changes and attempt to identify the pathogenesis of this disease. Forty-three ST dogs were enrolled on the basis of their breed, elevated plasma ALP activity and the presence of vacular hepatopathy in histological samples. Each dog underwent complete serum biochemistry and hematology testing, abdominal ultrasounds and liver biopsies. Overall, 54.6% of the dogs showed moderate to severe vacuolation. Almost 90% of the dogs exhibited no or minimal inflammation and extensive fibrosis was observed in 30% of the dogs. Neither systematization nor zonal distribution was observed regarding the location of the vacuolized hepatocytes. Vacuolated hepatocytes did not exhibit any PAS staining. Nine dogs had well-differentiated HCCA. Mitochondrial alterations were the most striking ultrastructural changes, with pleomorphic and enlarged mitochondria. In the STs of the present study, VH is a diffuse liver disease characterized by ballooned hepatocytes without glycogen content and exhibiting mitochondrial modifications. The two major hepatic complications described in the present cohort are fibrosis (30%) and neoplasia (21%).

Keywords: Vacuolation, liver, Scottish Terrier, histology

RESUME
Hépatopathie vacuolaire chez 43 Scottish Terriers français: étude morphologique

Les Scottish Terriers (ST) sont souvent atteints d’hépatopathie vacuolaire (HV), en association avec une élévation de l’activité des phosphatases alcalines (PAL), laquelle reflète possiblement une défaillance d’origine génétique affectant la stéroïdognèse. Cette défaillance pourrait également expliquer le développement de carcinome hépatocellulaire (CHC) chez cette race. Les objectifs de la présente étude étaient d’évaluer les lésions hépatiques dans une cohorte de ST français atteints de HV en utilisant l’histologie et la microscopie électronique à transmission (MET) dans le but de caractériser les modifications cellulaires et la pathogénèse de cette affection. Quarante-trois ST ont été inclus sur la base de leur race, d’une élévation de l’activité plasmatique de leurs PAL et de la présence d’hépatopathie vacuolaire à l’histologie. Pour chaque chien, un bilan biochimique et hématologique sanguin complet a été réalisé, ainsi qu’un examen échographique de l’abdomen et des biopsies hépatiques. Globalement, 54.6% des chiens montraient une vacuolisation modérée à sévère. L’inflammation était absente à minimale dans 90% des cas et une fibrose extensive a été observée chez 30% des chiens. Aucune systématisation ou distribution zonale n’a été notée concernant la localisation et la répartition des hépatocytes vacuolisés. Ces derniers ne présentaient pas la coloration PAS. Neuf chiens présentaient un CHC. Les modifications ultrastructurales les plus marquantes étaient les alterations mitochondriales, avec des mitochondries pleomorphiques et de taille élargie. Chez les STs de cette étude, l’hépatopathie vacuolaire se définissait comme une affection hépatique diffuse caractérisée par des hépatocytes ballonisés, sans contenu glycérogène et montrant des modifications mitochondriales. Les deux complications majeures observées au niveau du foie sont la fibrose (30%) et l’apparition de néoplasie (21%).

Mots-clés : Vacuolisation, foie, Scottish Terrier, histologie

Introduction
Scottish Terriers (STs) are known to develop vacuolar hepatopathy (VH) associated with elevated alkaline phosphatase (ALP) activity [3]. VH usually reflects hepatocellular cytosolic glycogen accumulation, confirmed with Periodic Acid Schiff (PAS) staining and easily differentiated from lipid vacuolation [4]. Hepatocytes are prominently swollen with clear and wispy cytoplasm and central nucleus [6, 4]. Distribution can be diffuse, zonal or involve individual cells. Discrete vacuoles are not observed unless concurrent lipid vacuolation exists, which actually makes the term “vacuolar” hepatopathy inappropriate [6, 4]. The origin of this hepatic disorder is currently unclear, and its pathogenesis remains undetermined [9, 20]. In a study conducted by Zimmerman et al., the authors hypothesized that increased ALP activity in STs is most likely attributable to non-clinical hyperadrenocorticism [34]. In a separate study, Sepesy et al. focused on disorders potentially associated with VH, and their results suggest an association between hepatic vacuolation, neoplasia, hepatobiliary diseases, and physiologic stress [28]. In a recent report, Center et al. concluded that high ALP activity and VH in STs likely reflects a genetic defect affecting steroidogenesis through an overproduction of androgenic hormones [3]. This result might also explain the appearance of hepatocellular carcinoma (HCCA) in these dogs.

We have described VH in STs in previous reports and have hypothesized that it could be a familial disease in this breed, with a heritable mode of transmission. If the light of this hypothesis, VH in STs might be independent of steroid
exposure and other endocrinian causes of elevated ALP activity, such as hyperadrenocorticism, diabetes mellitus or hypothyroidism [14]. In another report, we suggested a possible association between VH, hepatic fibrosis and HCCA [15]. On the basis of these findings, the aims of the present study were to describe histopathological characteristics of French STs presenting VH.

Materials and methods

ANIMALS

Dogs were enrolled both retrospectively and prospectively on the basis of their breed (they had to be purebred STs), elevated plasma ALP activity and the presence of VH in histological samples. They were also part of another study focusing on clinical, blood biochemical and hepatic histological data in French STs [23]. STs with VH were selected among these dogs to be included in the present cohort. In the previous study, dogs were grouped according to their ALP activity (normal, elevated, very elevated) and associations between clinical, biochemical and histological criteria were looked for. Forty-three pure-bred STs were included in the present study: 22 females (14 spayed) and 21 males (4 castrated). Ages ranged from 2 to 13 years (mean: 8.4). Twenty-two dogs were enrolled retrospectively and 21 were enrolled prospectively. Diagnosis of VH was based on the presence on liver biopsies of enlarged and swollen hepatocytes (so-called “vacuolated hepatocytes” in the present study as we describe features of a disease named “vacuolar hepatopathy”, although the term might be inappropriate) with clear cytoplasm, whatever their distribution (diffuse, zonal or individual).

BIOLOGICAL ANALYSIS

In the present cohort, ALP activity was between 161 and 8255 units/liter (U/L) with a mean of 1820 U/L. Dosage of ALP activity included all the iso-enzymes (bone, liver, intestine…) and was determined based on the laboratory reference interval (Idexx Alfort) for which the upper limit was set at 140U/L, based on internal studies conducted on a cohort of 100 healthy dogs receiving no medication, preliminary examined by a veterinarian.

The dogs of the present cohort presented to the Clinique des Cerisioz (Lyon, France) for different reasons (annual blood testing, digestive troubles, PUPD, lethargy, and/or dysorexia/anorexia). For each dog, a complete physical examination was performed and a detailed medical history was recorded. Previous medications and treatments that could have influenced hepatic metabolism (such as glucocorticoids or phenobarbital) lead to the exclusion of the dog. Each dog underwent serum biochemistry and hematology testing, abdominal ultrasounds and liver biopsies. Biochemistry panel included plasma or serum ALP, ALT, AST, cholesterol, GLDH, LDH, bilirubin, basal cortisol, basal bile acids, GGT, Total T4 and triglycerides [23]. Liver biopsies were performed on the basis of plasma ALP activity above 140U/L and presence of hepatic parenchymal modifications by ultrasound examination. Ultrasonographic criteria that raised the suspicion of vacuolar hepatopathy were hyperchoic, heterogenous and/or nodular hepatic parenchyma, with or without hepatomegaly. When hyperadrenocorticism, hypothyroidism and/or hepatic failure were suspected, based on compatible clinical signs (polyphagia, PUPD, abdominal ptosis, dermatologic changes, weight gain or loss…), hemotological and/or biochemical results (elevated liver enzymes activity, stress formula…) and ultrasound examination (bilateral adrenal enlargement, hepatomegaly, hepatic parenchymal modifications…), further examination was performed (ACTH stimulation test, low-dose dexamethasone suppression test, pre- and post-prandial serum bile acid analysis, thyroid hormone measurements, and/or additional imaging procedures) [8]. Dogs were excluded from the study if their medical history included medications that could have modified the biochemical results (especially plasma ALP activity) and/or liver histology and if their blood tests were compatible with conditions known to modify hepatic metabolism and histology (such as hyperadrenocorticism or diabetes mellitus).

METHODS

Histological procedures

Liver biopsies were made by the same ECVIM-CA diplomat, using a TruCut® (Coveto, France) biopsy needle (ultrasound guided) or realized surgically under laparotomy or coelioscopy (wedge biopsies). Biopsies were taken in area where changes in the hepatic parenchyma were most visible (either at ultrasound or directly visualized during laparotomy/coelioscopy). At least two samples were taken each time. All liver biopsies (either using a biopsy needle or wedge biopsies) were fixed in alcohol, formalin, and acetic acid, and embedded in paraffin (Paraplast X-TRA, Sigma-Aldrich, USA). Sections (3μm thickness) were deparaffinized, rehydrated and stained with HES for conventional histology, picrosirius red for analyzing fibrosis, PAS for analyzing glycogen, and Perl’s Prussian Blue for analyzing iron deposits. Copper overload was examined using rhodanin staining. Because of its cross-reactivity with canine species, immunohistochemical staining using specific antibodies against smooth muscle cells’ alpha-actin (Envision HRP K4000 kit, ref M0851, clone number 1A4, Dako, Denmark) was performed in order to identify activated hepatic stellate cells (responsible for fibrogenesis)[32].

Transmission electron microscopy procedures

Ten samples were fixed in 2% glutaraldehyde–0.1M Na-cacodylate/HCl buffer at a pH of 7.4 for 1 hour at 4°C, rinsed in 0.1M Na-cacodylate/HCl buffer; and post-fixed in 1% OsO₄–0.15M Na-cacodylate/HCl buffer at a pH of 7.4 for 1 hour at 4°C. Dehydration was performed using graded concentrations of ethanol, and samples were embedded in
Epon (EMbed 812 kit, Hatfield, UK). After polymerization and evaluation of areas of interest by semi-thin sections stained with toluidine blue, tissue was cut in ultra-thin sections, mounted on copper mesh grids, contrasted with uranyl acetate in methanol and lead citrate and examined with a transmission electron microscope (JEOL 1200EX, Peabody, USA) [11, 17].

Semi-quantitative evaluation of lesions

To ensure diagnostic consistency, histological sections (3µm thickness) were all read by the same blind observer, a hepatopathologist specializing in comparative pathology (MC). For each slide, the entire surface was read and each criterion was scored in a semi-quantitative way (vacuolation, activity, fibrosis). Two separate readings were done for each slide in order to refine the scores.

Hepatocellular vacuolation was scored from G1 to G3 (G1, or mild = <25% vacuolated hepatocytes; G2, or moderate = 26-50% vacuolated hepatocytes; G3, or severe = >50% vacuolated hepatocytes), according to the procedure in Center et al. [2]. Zonal location of vacuolated hepatocytes was also examined. The extent and pattern of fibrosis was evaluated using the METAVIR scoring system, in agreement with WSAVA guidelines on canine liver pathology [5, 26]. Fibrosis was scored from F0 to F4 (F0 = no fibrosis; F1 = portal fibrosis; F2 = moderate or minimally extensive fibrosis, starting to extend from portal areas to other zones of the hepatic lobule; F3 = extensive fibrosis, present in all the different zones of the hepatic lobule; F4 = cirrhosis, i.e., severe fibrosis with nodular regeneration). Hepatocellular apoptosis, necrosis, and hepatic inflammation (collectively referred to as activity) were scored from A0 to A3 (A0 = piecemeal and lobular necrosis absent; A1 = minimal piecemeal necrosis associated with absent to severe lobular necrosis, or absent piecemeal necrosis associated with moderate to severe lobular necrosis; A2 = moderate piecemeal necrosis associated with absent to severe lobular necrosis; A3 = severe piecemeal necrosis associated with absent to severe lobular necrosis) using the METAVIR scoring system (Table I). Pre-neoplastic and neoplastic lesions (cell dysplasia, HCCA) were also looked for and identified [33]. The same procedure was performed for non-cirrhotic architectural modifications, such as nodular regenerative hyperplasia (NRH) [10].

RESULTS

LIVER BIOPSIES

Fifty-five liver biopsies were performed: 39 using the biopsy needle and 16 wedge biopsies. The number of biopsies does not correspond to the number of dogs, as some dogs had both wedge biopsies and biopsies performed using the biopsy needle. For dogs that had both types of biopsies, samples were evaluated together as one single entity.

HISTOPATHOLOGIC FINDINGS

Vacuolated hepatocytes were swollen and clear, with small nuclei transposed to the periphery of the cell (Figure 1.A1). No inflammatory cells were present. Nineteen dogs (44%) showed mild vacuolation (G1), and 24 dogs (56%) exhibited moderate to severe vacuolation (12 dogs G2 and 12 dogs G3) (Table 2). No systematization was observed regarding the location of the vacuolized hepatocytes, with no zonal distribution. Vacuolated hepatocytes did not exhibit any PAS staining, in contrast with adjacent non-vacuolated

<table>
<thead>
<tr>
<th>Vacuolation</th>
<th>Fibrosis</th>
<th>Activity</th>
<th>Lobular necrosis</th>
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<tbody>
<tr>
<td>G1: mild (&lt;25%)</td>
<td>F0: absent</td>
<td>A0</td>
<td>Absent/Minimal</td>
</tr>
<tr>
<td>G2: moderate (26-50%)</td>
<td>F1: portal fibrosis</td>
<td>A1</td>
<td>Moderate</td>
</tr>
<tr>
<td>G3: severe (&gt;51%)</td>
<td>F2: minimally extensive fibrosis</td>
<td>A1</td>
<td>Severe</td>
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Table I: Semi-quantitative evaluation of vacuolation, fibrosis and activity

<table>
<thead>
<tr>
<th>Grade</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
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<tbody>
<tr>
<td>Dogs nb (%)</td>
<td>19 (44%)</td>
<td>12 (28%)</td>
<td>12 (28%)</td>
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Table II: Number and percentage of dogs with hepatocellular vacuolation, activity, and fibrosis.
VACUOLAR HEPATOPATHY IN 43 SCOTTISH TERRIER DOGS

Regarding activity (i.e., inflammation and necrosis), piecemeal/lobular necrosis and chronic portal inflammation (Figure 1.A2) were observed in approximately 50% of the dogs: 16 dogs (38%) had minimal activity, and only five dogs (11%) showed moderate to severe activity (Table 2).

Liver fibrosis was present in portal and periportal areas (Figure 1.B1) and cirrhosis was noticed in 6 animals (Figure 1.B2). Nineteen (44%) dogs showed no significant fibrosis (F0+F1), 10 dogs (23%) had minimal fibrosis (F2), 8 dogs (19%) had extensive fibrosis (F3) (Table 2).

ADDITIONAL HISTOLOGIC FINDINGS

Three dogs had a replacement of the normal hepatic parenchyma by variably sized nodules that contained alternating areas of atrophy and hypertrophy, with minimal to no fibrosis, resembling human nodular regenerative hyperplasia (Figure 3.A). Three additional dogs had histologic changes consistent with a pre-neoplastic lesion identified as small cell dysplasia (Figure 3.B1). Nine dogs had well-differentiated hepatocellular carcinoma (Figure 3.B2), 4 of which also had significant fibrosis (F3/F4).

IRON AND COPPER

The iron overload commonly observed in canine macrophages was present. No copper overload was noted.
IMMUNOHISTOCHEMICAL FINDINGS

The expression of smooth muscle α-actin was increased around ballooned hepatocytes (Figure 4.A) and in fibrotic areas in dogs with moderate to severe fibrosis (Figure 4.B).

ELECTRON MICROSCOPY FINDINGS

Hepatocytes with ultrastructural changes were usually scattered among normal adjacent cells (Figure 5.A1). No canicular or nuclear changes were observed within hepatocytes ultrastructurally. Hepatocytes had optically empty areas in their cytoplasm, pushing aside cytoplasmic organelles and making them appear clustered around the nucleus or close to cytoplasmic membrane. The cytosol contained large clear spaces with few organelles and lacked normal glycogen rosettes (Figure 5.A2). The lack of glycogen within affected hepatocytes was confirmed through PAS staining, without any evidence of cytoplasmic glycogen rosettes (Figure 2).

Figures 3: Precancerous and cancerous lesions in Vacuolar Hepatopathy. A; after picrosirius red staining. Formation of nodules formed by two or more cell-thick hepatocyte cords that lack normal lobular architecture, resembling nodular regenerative hyperplasia in humans (X10). Note the lack of extensive fibrosis and the presence of small remaining parenchymal areas with atrophic hepatocytes (arrow). B; after HES staining. B1; Presence of small cell dysplasia in the upper right (SCD), consisting of basophilic hepatocytes arranged in one to two cell-thick cords (X20). Note vacuolated hepatocytes (VH) on the lower left. B2; Hepatocellular carcinoma (HCC), characterized by hepatocytes irregularly arranged in cords of varying thickness and the presence of gland-like spaces (X10).

Figures 4: Activation of stellate cells labeled by immunohistochemistry (PO) using alpha smooth muscle actin antibody. A; Normal (black arrows) and ballooned hepatocytes (white arrows). Ballooned hepatocytes are surrounded by activated stellate cells immunoreactive to alpha smooth muscle actin (arrow heads), forming a delicate continuous network (X20). B; Abundant network of activated stellate cells immunoreactive to alpha smooth muscle actin (arrows) surrounding regenerative nodules (*) in a dog with cirrhosis (X5). Note the residual portal tract (PT).
Mitochondrial alterations were the most striking ultrastructural changes (Figure 5.B1), with pleomorphic and enlarged mitochondria showing highly disorganized matrix substance and loss of matrix dense bodies (Figure 5.B2), with abnormally long and thick crests and black crystallin internal inclusions.

**Discussion**

The objective of the present study was to assess the hepatic lesions in a cohort of French STs with VH using histological techniques and TEM in order to characterize cellular changes and attempt to identify the pathogenesis of this disease, in the hope that understanding of the observed ultrastructural changes might highlight the mechanisms underlying hepatocytic modifications.

Canine VH, first described by Center et al. in 1996 [2], is cytologically characterized by enlarged hepatocytes, rarified cytosol and increased hepatocyte fragility [4]. The term VH is used inconsistently, but usually reflects hepatocellular cytosolic glycogen accumulation, visible microscopically as tiny rosettes lacking membrane confinement [4]. As described by Center et al., VH is characterized by discrete vacuoles, unless concurrent lipid vacuolation exists, particularly in STs with VH (mixture of glycogen and lipids) [4]. In the present study, vacuolation was mild (G1) in 45.4% of the population.
studied, while 54.6% of the dogs showed moderate to severe vacuolation (G2-G3). No zonal distribution was observed for vacuolated cells. In several papers, the clear aspect of the affected hepatocytes was described as being glycogen [1, 4, 7, 24]. Interestingly, in our studies, the glycogen cell content was evaluated for all liver samples using PAS staining and was not shown to be increased in affected cells. Indeed, no rosette-like features were observed in the cytoplasm of altered hepatocytes, unlike in the adjacent normal hepatocytes. This observation was confirmed by TEM, which showed a ballooned aspect of the affected hepatocytes, with a reticulated cytoplasm instead of vacuoles, making the term “vacuolar” potentially unsuitable in the present study. The difference observed between our study and previous reports may be explained by differences in both methodologies but as glycogen was objectivated in normal adjacent hepatocytes, it seems reasonable to assume that glycogen would also have been detected in ballooned hepatocytes if present. In the same way, previous studies reported that the nucleus of the hepatocytes was central in VH [4, 6] while in the present study, the nucleus was displaced to the cell periphery. These observations raise the question of whether VH in STs from the US and STs from France is the same disease or not.

In light of the observations of the present study, VH in French STs can be described as a diffuse liver disease characterized by ballooned hepatocytes without glycogen content and exhibiting mitochondrial modifications. The two most frequent associated lesions reported in the present study were fibrosis and neoplasia, leading the authors to consider VH as a potential cause. Although almost 90% of the dogs exhibited no or minimal activity (apoptosis, necrosis, and/or inflammation), extensive fibrosis was observed in 30% of the animals. Three dogs had architectural abnormalities consistent with nodular regenerative hyperplasia (NRH), characterized by an abnormal distribution of vessels leading to parenchymal reorganization in nodules without any fibrotic process [10]. Other concurrent hepatic lesions in this study were small cell dysplasia (3 dogs) and cirrhosis (6 dogs), which are thought to be pre-cancerous lesions [7, 25, 33]. HCCA was observed in 9 dogs (21%) and was associated with cirrhosis in 4 of them. This last observation is in agreement with findings described by Center et al., even if the percentage of dogs with VH and HCCA is slightly lower (30% in Center’s study versus 21% in the present study) [4].

To corroborate Center’s observations, in a previous study, we found a significant association (p<0.05) between ALP activity and vacuolation of liver parenchyma [23] which is also in agreement with a previous report conducted on 336 dogs with histologically confirmed VH [4, 28]. In the same previous study, we also found significant association between ALP activity and presence of HCCA, with ALP activity being strongly correlated with HCCA. These results support the association between ALP activity, VH and HCCA.

Several studies have implicated glucocorticoid metabolism in the development of the disease, but little is actually known about the real mechanism involved [24, 28, 34]. It is interesting to note that in the present study, vacuolated hepatocytes showed common features with hepatocytes of dogs presenting hyperadrenocorticism (which is a dysfunction in the glucocorticoid metabolism), such as cytoplasmic and mitochondrial changes. Similarly to what is seen in canine hyperadrenocorticism, the cytosolic optically empty areas seemed to push aside cytoplasmic organelles, making them appear clustered around the nucleus or close to cytoplasmic membrane, and some mitochondria were giant and of irregular form, with abnormally long and thick crests and black crystallin internal inclusions.

In human pathology, mitochondrial disorders are observed in hereditary diseases termed “mitochondrial cytopathies”[1,19]. Liver tissue can be predominantly affected, leading to hepatic failure through development of fibrosis. The common pathogenesis in this spectrum of diseases is a defect in the mitochondrial respiratory chain, leading to oxidative stress. Mitochondrial injuries described in these mitochondrialopathies share similarities with those observed in the canine liver samples studied using TEM. Interestingly, Human Non Alcoholic Steato-Hepatitis (NASH) shares some features with vacuolar hepatopathy as described in the present study [29,30]. Murine models demonstrated that a decrease in hepatic fatty acid oxidation associated with a decrease in mitochondrial enzyme activity precedes the development of NASH [12,27]. These observations suggest that a disruption in mitochondrial ultrastructure and metabolism could be responsible for the development of VH. Regarding the shape of hepatocytes in VH, modifications of the cytoskeletal intermediate filaments K8 and K18 in response to oxidative stress could be responsible for the ballooning of the cells as demonstrated in murine models [13,21].

Paradis et al. demonstrated an association between NASH and HCCA, raising the hypothesis that NASH could pave the way for an alternative manner of carcinogenesis, allowing HCCA to develop in non-cirrhotic hepatic parenchyma, contrary to what is found in most human liver diseases. [22, 25]. This process could be another similarity between NASH and VH, as in the present study, among the 9 dogs that developed HCCA, 5 exhibited no signs of cirrhosis.

The present study has several limitations. Liver biopsies were not performed in the same manner for all dogs (either needle biopsies or wedge biopsies) and this might have been a source of bias in the interpretation of liver histology. Wedge biopsies provide a more accurate view of most liver disorders than needle biopsies but needle biopsies are sufficient for many approaches, since samples from more than one liver lobe are provided [6]. However, to prevent such a bias, groups could have been made separating dogs according to the way biopsies had been realized (needle or wedge biopsies). For dogs that had both types of biopsies, samples were evaluated as a single entity while it might have been interesting to make comparisons in order to see if the way biopsies were made had an incidence on histological results. For cost reasons,
only 10 cases were studied using TEM, which is not much given the total number of dogs (43). Studying all patients with TEM would have provided a larger view of the hepatic lesions. The fact that histological samples were read by a non-veterinarian, non-boarded pathologist can be seen as a limitation; however, in the present study, the pathologist who read the slides is an international reference on human liver histology and is also very adept at analyzing canine histological samples, having worked on numerous studies involving comparative pathology.[16,18, 31] Regarding the way hepatic lesions were graded, the META VIR scoring system was used to characterize activity and fibrosis in the present study. This scoring system was initially built for the evaluation of human chronic hepatitis and is routinely used in human liver histology. We carefully considered the WSAVA guidelines regarding the evaluation of liver biopsies and the features of the META VIR scoring system fit all the criteria and recommendations listed by the liver study group [5].

In the present study, VH in French STs was characterized by different grades of hepatocyte vacuolation and fibrosis. Hepatic lesions showed little inflammation and/or necrosis. Interestingly, vacuolation was not associated with glycogenic content, contrary to what was described in previous reports. These observations as well as our TEM findings tend to describe the affected hepatocytes as “ballooned” rather than vacuolated cells. The similarities between human NASH and VH characteristics described in the present study are striking, and using NASH as a model for canine VH may be helpful in investigating the etiology, prevention and possible treatments of this disease. Many molecular patterns remain to be investigated in order to understand the underlying mechanisms that trigger the development of VH, especially the role of keratin filaments and mitochondrial abnormalities.

References


VACUOLAR HEPATOPATHY IN 43 SCOTTISH TERRIER DOGS


