

# Clinical, blood biochemical and hepatic histological data in 49 French Scottish Terriers dogs according to their plasma ALP activity, hepatic vacuolation and the presence or absence of hepatocellular carcinoma

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## SUMMARY

The present study aims at describing the clinical, blood biochemical and hepatic histological features of French Scottish Terrier dogs and finding associations between these different data.

Dogs were divided into 3 groups, based on their plasma ALP activity (control, elevated, very elevated). Hepatic biopsies were graded according to their degree of vacuolation, fibrosis, inflammation (activity) and the presence or absence of hepatocellular carcinoma (HCCA). Correlations between clinical, blood biochemical and histological features were examined and comparisons were made with the control group.

Forty-nine dogs were included in the study, 18 males and 31 females, with a mean age of 8.3. For most of the dogs, hepatic vacuolation and fibrosis were, respectively, moderate and mild, and inflammation (activity) was low. Plasma ALP activity was significantly associated with hepatic vacuolation ( $p=0.020$ ). Fibrosis was positively correlated with age, plasma ALT and AST activities. Inflammation was positively correlated with plasma ALT, AST activities and cholesterol. Vacuolation was positively correlated with age, ALT activity and cholesterol. Seventy-five percent of dogs with HCCA had a G3 vacuolation grade, and ALP activity was significantly higher in dogs with HCCA.

In the present cohort, elevated plasma ALP activity was associated with hepatic vacuolation and HCCA. Hepatic vacuolation was frequent but moderate (31.9% minimal, 23.4% mild, 23.4% moderate, 10.6% severe). Plasma ALT activity was found to be a good indicator of hepatic vacuolation, fibrosis and inflammation. There is a potential association between hepatic vacuolation and HCCA.

**Keywords :** Vacuolation, liver, dog, ALP, hepatocellular carcinoma

## RESUME

**Données cliniques, biochimiques sanguines et histologiques chez 49 Scottish Terriers français en fonction de l'activité plasmatique des PAL, de la vacuolisation hépatique et de la présence ou non d'hépatocarcinome.**

L'objectif de cette étude est de décrire les caractéristiques cliniques, biochimiques sanguines et hépato-histologiques d'une cohorte de Scottish Terriers français en essayant de trouver des associations potentielles entre ces différentes données.

Les chiens ont été divisés en 3 groupes sur la base de l'activité plasmatique de leur PAL (contrôle, élevée, très élevée). Les biopsies hépatiques ont été évaluées en fonction de leur degré de vacuolisation, inflammation (appelée activité), fibrose et de la présence ou non d'hépatocarcinome. Une recherche de corrélation entre les entités cliniques, biochimiques sanguines et histologiques a été effectuée et des comparaisons ont été faites avec le groupe contrôle.

Quarante-neuf chiens ont été inclus dans l'étude, 18 mâles et 31 femelles, avec un âge moyen de 8,3 ans. Chez la plupart des chiens, la vacuolisation et la fibrose étaient respectivement modérée et faible, et l'inflammation était également faible. L'activité plasmatique des PAL était significativement associée avec la vacuolisation ( $p=0,020$ ). La fibrose était corrélée positivement avec l'âge et l'activité des ALAT et des AST. L'inflammation était corrélée positivement avec l'activité des ALAT, des AST et le cholestérol plasmatiques. La vacuolisation était corrélée positivement avec l'âge, l'activité des ALAT et le cholestérol. Soixante-quinze pourcent des chiens présentant un hépatocarcinome avaient une vacuolisation de grade G3 et l'activité des PAL était significativement plus élevée chez les chiens avec hépatocarcinome.

Dans la présente étude, une activité plasmatique des PAL élevée semble associée avec la vacuolisation hépatique et la présence d'hépatocarcinome. La vacuolisation hépatique est fréquente mais plutôt modérée. L'activité plasmatique des ALAT se révèle un bon indicateur de vacuolisation, fibrose et inflammation hépatique. Il existe une association potentielle entre la vacuolisation hépatique et la présence d'hépatocarcinome.

**Mots-clés :** Vacuolisation, foie, chien, PAL, hépatocarcinome

## Introduction

Diagnosis of vacuolar hepatopathy (VH) is common in the dog; one retrospective study characterised this lesion in approximately 17% of 500 sequential canine biopsies [3]. However, this frustrating morphologic diagnosis is often insufficient to render a definitive causal diagnosis, and the lesion is, at best, considered a "syndrome". 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 [19]. Indeed, stress induced by a disease such as neoplasia could be responsible for the release of an endogenous steroid other than cortisol, leading to the development of VH.

While any dog breed, or any mix of dog breeds, can develop a degenerative VH, Scottish Terriers have a high risk [3]. The origin of this hepatic disorder is currently unclear, and its pathogenesis remains undetermined [11, 16]. Dogs with VH secondary to exogenous glucocorticoid administration or high endogenous glucocorticoid concentration typically have clinical signs of glucocorticoid excess (polyphagia, polyuria-polydipsia...) and high serum or plasma ALP activities [19]. A pioneering study explored the association between high ALP activity and increased adrenal steroid hormone concentrations in apparently healthy Scottish Terriers, and the authors hypothesised that increased ALP activity in Scottish Terriers is most likely attributable to non-clinical hyperadrenocorticism [20]. A breed-related predisposition to VH and to hepatocellular carcinoma (HCCA) has also been described, but clinicopathological features between Scottish Terriers presenting for VH with or without HCCA show only a few significant predictive differences [3].

On the basis of these observations, the goals of the present study were to describe the hepatic lesions in a cohort of French Scottish Terrier dogs with different values of plasma ALP activity (in the reference interval, elevated, very elevated), to study associations and correlations between ALP activity and hepatic modifications and to find other clinical or biochemical features that could also be associated with these hepatic modifications. The objectives were also to compare the dogs' ALP activity and the presence or absence of HCCA from a clinical, biochemical and histological point of view to find markers and/or potential predictors of severity regarding hepatic histological modifications.

## Materials and methods

### PATIENT SELECTION

The dogs enrolled in the study were selected from among Scottish Terriers that came to the Clinique des Cerisiers between April 2005 and May 2011. Dogs were recruited both prospectively and retrospectively. To be included in the study, participants had to be purebred Scottish Terriers and free of any disease (other than HCCA) or medication known to potentially influence ALP activity. Scottish Terriers with plasma ALP activity in the reference interval were used as control dogs. All dogs were first seen in consultation for various reasons (vaccination, annual check-up, polyuria-polydipsia syndrome (PU/PD), vomiting, etc.) and underwent a thorough examination. Body condition scores were assigned on a scale from 1 to 5/5 (1: emaciated, 2: skinny, 3: normal, 4: overweight, 5: obese) and clinical signs were classified using a clinical scoring system from 1 to 3: 1 when asymptomatic, 2 when symptomatic but well (PU/PD alone) and 3 when symptomatic and ill (vomiting, diarrhoea, lethargy, anorexia, weight loss, etc.). History and previous medications were carefully recorded, and dogs that had received drugs known to modify liver metabolism such as phenobarbital or glucocorticoids were excluded from the study. A wide biochemical blood profile was performed for

each dog. In cases where hyperadrenocorticism, diabetes mellitus or a hepatic anomaly such as a porto-systemic shunt was suspected, additional appropriate testing was performed to rule out any of these diseases, or to exclude the dog from the study, before performing liver biopsies. All dogs had their serum basal cortisol measured, and an ACTH stimulation test and/or low-dose dexamethasone test (LDDT) was performed if clinical signs, history and basal cortisol were compatible with hyperadrenocorticism. Pre-prandial serum bile acids were only measured when there was a clinical and biochemical suspicion of hepatic failure, porto-systemic shunt or any other hepatic vascular anomaly. Dogs that were suspected to have extra-hepatic neoplasia (due to clinical signs such as intra-abdominal mass(es) seen at ultrasound examination or polyadenomegaly) were excluded from the study, but dogs presenting with HCCA were not excluded to look for potential associations between HCCA and the biochemical/histological features observed. The upper limit of ALP reference interval with the method used was 140 U/L and dogs with values below 140 U/L were assigned to the control group. ALP activity between 140 and 1000 U/L was considered elevated, and ALP activity over 1000 U/L was considered highly elevated, based on the laboratory reference interval. Abdominal ultrasounds and liver biopsies were completed for each dog.

### PROTOCOLS

Venepuncture was performed at the jugular vein for each dog, after hair had been clipped and the skin cleaned. Blood was immediately transferred into separate tubes containing sodium-heparin, EDTA and a tube containing no anti-coagulant. Tubes were then centrifugated to isolate plasma and serum. Specimens were stored between 4 and 8°C and sent to Idexx Laboratory (Alfort), where blood cholesterol, ALP, ALT, GGT, AST, GLDH, total T4 (TT4), bilirubin, triglycerides, LDH, pre-prandial bile acids and basal cortisol were measured. Blood analysis was performed on heparinized plasma or serum, except for bile acids (EDTA plasma or serum required). Biochemical variables were measured using a colorimetric Modular P analyzer distributed by Roche (Randox for bile acids), with ThermoFisher reagents. Cortisol dosage was realized with a technique based on immunoenzymology (ELISA), using an Immulite 2000 analyzer from Siemens. All analyzers were daily calibrated using at least 2 levels of control. As for all variables, the laboratory's reference interval and especially the upper limit used for ALP activity (140 U/L) were statistically determined after an internal study was conducted on a cohort of 100 healthy dogs receiving no medication, preliminarily examined by a veterinarian. The value of 1000 U/L for ALP activity was arbitrarily set, based on the experience of the authors and the laboratory, in order to separate dogs into different groups (controls, elevated ALP activity, very elevated ALP activity).

Ultrasound examination and liver biopsies were made by the same ECVIM-CA diplomate for each candidate. Biopsies were done using a TruCut® device or were made

surgically if the dog underwent laparotomy or coelioscopy. The liver biopsies were fixed in AFA (alcohol, formalin, and acetic acid) and embedded in paraffin wax. Sections (3 µm thickness) were deparaffinised, rehydrated and stained with HES (haematoxylin-eosin-safran) for conventional histology, picosirus red for highlighting fibrosis, periodic acid-Schiff (PAS) for highlighting glycogen and Perl's for highlighting iron deposits. Histological study of the liver specimens was performed blindly by the same hepatopathologist, and the hepatic lesions were described according to their degree of activity (i.e., necrosis and inflammation), fibrosis and vacuolation (Tab. I). Hepatocellular vacuolation was scored from G0 to G4 (G0: absent; G1: minimal; G2: mild = <25% vacuolated hepatocytes; G3: moderate = 26-50% vacuolated hepatocytes; G4: severe = >51% vacuolated hepatocytes), based on the grading scheme described by Center [1]. Zonal location of vacuolated hepatocytes was also examined. The extent and pattern of fibrosis was evaluated using the METAVIR scoring system, which fits the criteria of the World Small Animal Veterinary Association (WSAVA) guidelines on canine liver pathology [7, 18]. 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 of 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, also using the METAVIR scoring system (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) [18].

**STATISTICAL ANALYSIS**

A descriptive analysis of the population was first realised. Qualitative variables were expressed using percentages and absolute numbers or ratios taking into account the number of dogs for which results were available (when applicable). Quantitative variables were described using medians, maximums and minimums. Qualitative variables that were potentially associated to severe vacuolation (G3/G4), fibrosis (F3/F4) or cancer were studied using Pearson's chi-square

test or Fisher's exact test. Quantitative variables were studied using Mann-Whitney's test. This non-parametric test was used rather than a variance analysis because of the small numbers of dogs. For ordinal variables, a chi-square test for trend was used. Statistical correlations between clinical, histological and biochemical features were studied using Student's correlation test (*t*-test). A ROC curve analysis was realized to evaluate the sensitivity and specificity of biochemical variables as markers of vacuolation. The threshold giving the best compromise in terms of sensitivity and specificity was determined as the point located at the highest left upper corner of the curve. Statistical analysis was performed using the 17.0 version of the SPSS software for Windows and the 3.0.1 version of the R software.

**Results**

**POPULATION**

Preliminarily, 68 French Scottish Terrier dogs were enrolled in the study. Of these, 19 were excluded because of incomplete clinical files or concurrent diseases that could have interfered with the results such as hypothyroidism, hyperadrenocorticism, diabetes mellitus or extra-hepatic neoplasia. These exclusions left 49 dogs in the study population, including 18 males (36.7%) and 31 females (63.3%). Among the males, 1 was castrated, and 12 females were spayed. Ages ranged from 1 to 13 years (mean: 8.3±2.9; median: 8.5). Regarding clinical signs, 18 dogs had a clinical score of 1 (36.7%), 15 had a clinical score of 2 (30.6%) and 16 had a clinical score of 3 (32.7%). The body condition score was 2/5 for 6 dogs (12.2%), 3/5 for 27 dogs (55.1%), 4/5 for 13 dogs (26.5%) and 5/5 for 3 dogs (6.1%). Five dogs (10.2%) had plasma ALP values below 140 U/L and were used as control dogs, 20 (40.8%) had ALP values between 140 and 1000 U/L (group 1) and 24 (49%) had their ALP activity above 1000 U/L (group 2). Among the control dogs, 2 (40%) had a clinical score of 2 (PU/PD alone). Among the dogs from the group 1, 8 (40%) had a clinical score of 1 (asymptomatic), 6 (30%) had a clinical score of 2 and 6 (30%) had a clinical score of 3 (ill). In group 2, 7 (29.2%) dogs had a clinical score of 1, 7 (29.2%) had a clinical score of 2 and 10 (41.6%) had a clinical score of 3.

**HISTOLOGY**

When hepatocytic vacuolation was present, vacuolated cells were swollen and clear, with small nuclei transposed to

VACUOLATION	FIBROSIS	ACTIVITY	Lobular necrosis		
			Absent/Minimal	Moderate	Severe
G0: absent	F0: absent	<i>Piecemeal</i>	Absent	A0	A1
G1: minimal(rare vacuolized hepatocytes)	F1: portal fibrosis	<i>necrosis</i>	Minimal	A1	A1
G2: mild (<25%)	F2: minimally extensive fibrosis		Moderate	A2	A2
G3: moderate (26-50%)	F3: extensive fibrosis		Severe	A3	A3
G4: severe (>51%)	F4: cirrhosis				

TABLE I: Semi-quantitative evaluation of vacuolation, fibrosis and activity.

the periphery of the cell. No inflammatory cells were present. Neither systematisation nor zonal distribution was observed regarding the location of the vacuolised hepatocytes within the hepatic lobules.

Vacuolation was graded in 47/49 dogs (data regarding vacuolation were lacking for 2 dogs). It was absent to mild (G0 to G2) in 31 dogs (66%) and moderate to severe (G3 to G4) in 27 dogs (34%) with 5 dogs (10.6%) graded as G0; 15 (32%) as G1; 11 (23.4%) as G2; 11 (23.4%) as G3 and 5 (10.6%) as G4. Overall, vacuolation was mild to moderate, with 78.7% of the dogs graded G1 to G3. Activity was graded A0 in 34 dogs (69.4%), A1 in 11 dogs (22.4%), A2 in 3 dogs (6.2%) and A3 in 1 dog (2%). Fibrosis was graded in 48/49 dogs (data regarding fibrosis were lacking for 1 dog). It was mild (F0 to F2) in 39 dogs (81.2%) and moderate to severe (F3 to F4) in 9 dogs (18.8%) with 22 dogs (45.8%) F0; 10 dogs (20.8%) F1; 7 dogs (14.6%) F2; 3 dogs (6.3%) F3 and 6 dogs (12.5%) F4 (Tab. II). Six dogs (12.5%) had HCCA.

## CLINICAL/BIOCHEMICAL FEATURES AND VACUOLATION

There were 54.5% of male dogs who had a vacuolation grade of G3 to G4, versus 26.7% of females. Despite this finding, no significant association was found between vacuolation and sex (male versus female, castrated versus non-castrated). In the same way, vacuolation was not found to be associated with body condition scores nor clinical scores, but was on the contrary significantly associated with age ( $p=0.022$ ), with a positive correlation found between vacuolation score  $\geq G3$  and age. Vacuolation was also significantly associated plasma ALP activity ( $p=0.020$ ) but no significant correlation was found between these two variables ( $p=0.11$ ). Among the dogs from the control group, the majority (50%) of the dogs had their vacuolation graded G1, and none had a vacuolation grade of G3/G4. Of the dogs from group 1, 20% had a vacuolation grade of G3/G4, with a majority of G1 (40%) and G2 (30%) grades. In group 2, the majority (55%) of the dogs had a vacuolation grade of G3/G4 (Fig. 1). The ROC curve analysis showed that plasma ALP activity was an interesting marker of moderate to severe vacuolation (G3-G4) and that the most discriminating cut-off value was 1030 U/L (Fig. 2). Using this cut-off, sensitivity was 75% and specificity was 68%, with an area under the curve of 0.710 and a 95% confidence interval of 0.557-

0.862. Vacuolation was also significantly associated with ALT activity ( $p=0.016$ ), and a positive correlation was found between ALT activity and vacuolation  $\geq G2$ . The ROC curve analysis showed that the cut-off value of 246 U/L had the best sensitivity and specificity. Pre-prandial serum bile acids and plasma cholesterol were also positively correlated with vacuolation  $\geq G2$  ( $p=0.041$ ).

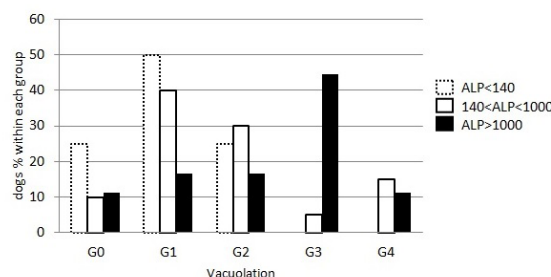


FIGURE 1: Semi-quantitative evaluation of vacuolation, activity and fibrosis in 49 French Scottish Terriers according to their plasma ALP activity. Control dogs with plasma ALP activity <140 U/L are represented with a dotted line box, dogs with ALP activity between 140 and 1000 U/L with a full line box and dogs with ALP activity >1000 U/L with a black box.

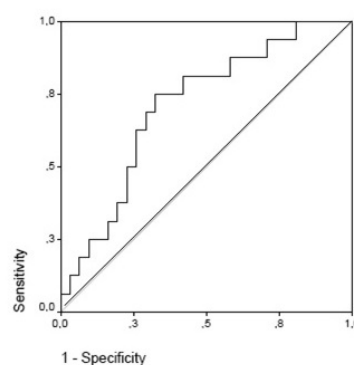


FIGURE 2: Sensitivity and specificity of plasma ALP activity regarding severe vacuolation (G3/G4). The area under the curve is 0.710,  $p=0.020$ .

## CLINICAL/BIOCHEMICAL FEATURES AND INFLAMMATION (ACTIVITY)

Plasma ALT and AST values were significantly associated with histological activity of the lesions ( $p=0.049$  and  $0.045$ , respectively). A positive correlation was found between these 2 variables and activity  $\geq A2$ . Plasma cholesterol was also

Vacuolation	Grade	G0	G1	G2	G3	G4
	Dogs nb (%)		5(10,6)	15 (32%)	11 (23,4%)	11 (23,4%)
Activity (piecemeal, lobular necrosis, portal inflammation)	Grade	A0	A1	A2	A3	
	Dogs nb (%)	34 (69,4%)	11 (22,4%)	3 (6,2%)	1 (2%)	
Fibrosis	Grade	F0	F1	F2	F3	F4
	Dogs nb (%)	22 (45,8%)	10 (20,8%)	7 (14,6%)	3 (6,3%)	6 (12,5%)

TABLE II: Number and percentage of dogs with hepatocellular vacuolation, activity, and fibrosis.

significantly associated with activity ( $p=0.042$ ) and positively correlated with activity  $\geq A3$ .

**CLINICAL/BIOCHEMICAL FEATURES AND FIBROSIS**

Castrated/spayed dogs had significantly more severe fibrosis (42% F3/F4) than non-castrated/spayed dogs, with only 11% F3/F4 ( $p=0.019$ ). Age and fibrosis were positively correlated for a fibrosis grade  $\geq F2$ . Fibrosis was not associated with body condition scores nor clinical scores but was significantly associated with plasma ALT activity ( $p=0.003$ ), and there was a positive correlation between ALT activity and fibrosis  $\geq F2$ . Fibrosis was also significantly associated with plasma AST activity ( $p<0.001$ ) with a positive correlation between AST activity and fibrosis  $\geq F2$ . The ROC curve analysis showed that using a cut-off value of 82 U/L, sensitivity was 89% and specificity was 90%, with an area under the curve of 0.921 and a 95% confidence interval of 0.820-1 (Fig. 3).

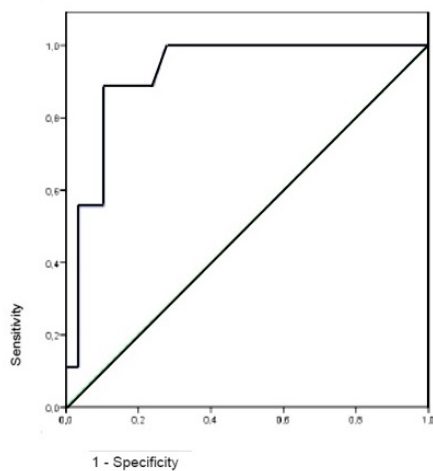


FIGURE 3: Sensitivity and specificity of plasma AST activity regarding fibrosis. The area under the curve is 0.921,  $p<0.001$ .

**HCCA AND CLINICAL/BIOCHEMICAL/HISTOLOGICAL FEATURES**

All the dogs with HCCA belonged to group 2, and plasma ALP activity was positively correlated with the presence of HCCA (Fig. 4). ALP and ALT activities were significantly associated with the presence of HCCA ( $p= 0.016$  and  $0.002$ , respectively), as were pre-prandial serum bile acids, basal cortisol and plasma bilirubin ( $p= 0.003$ ,  $0.005$  and  $0.013$ , respectively). The presence of HCCA was not correlated with age. Sixty-seven percent of the dogs with HCCA had a vacuolation grade of G3 (4/6 dogs), while the majority of dogs without HCCA (excluding control dogs) had vacuolation grades of G1 (32%) or G2 (24%). In the 6 dogs with HCCA, activity was quite mild (2 dogs graded A0, 2 dogs A1) and 2/6 dogs had cirrhosis (F4).

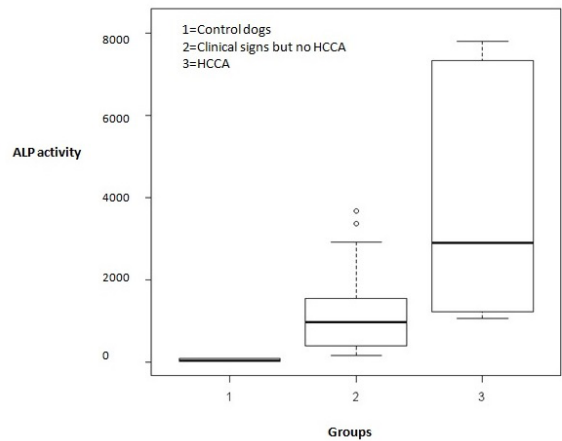


FIGURE 4: Plasma ALP activity in control dogs versus dogs without hepatocellular carcinoma and dogs with hepatocellular carcinoma. Group 1: Control dogs; group 2: Dogs with clinical signs

**Discussion**

Interestingly, clinical scores were not associated with histological features in the present study, which could mean that clinical signs are not good indicators of the severity of hepatic lesions. However, 41.6% of the dogs from group 2 (ALP activity  $\geq 1000$  U/L) had a clinical score of 3 (10/24 dogs), which is worth noting. Neutering was associated with fibrosis, with more important fibrosis in castrated/spayed dogs. Age was correlated with fibrosis and vacuolation, which could be expected on the assumption that hepatic vacuolation progresses for the dog's entire life.

Overall, vacuolation was mild to moderate, with 78.7% of the dogs graded G1 to G3 (32% G1, 23.4% G2, and 23.4% G3). Activity was minimal (69.4% A0 and 22.4% A1), and fibrosis was also mild (81.2% F0-F1-F2). HCCA was present in only 6/49 dogs.

Plasma ALP activity was positively correlated with the presence of HCCA and significantly associated with vacuolation, although no positive correlation could be found between ALP activity and vacuolation. The  $p$  value was 0.11, which indicates that if a bigger cohort of dogs was used, significance might have been reached, although this statement remains purely speculative.

In the present study, correlation tests showed that plasma ALT was a very good marker for fibrosis, activity and vacuolation. ALT might be an interesting variable to consider when vacuolar hepatopathy is suspected in Scottish Terriers. Plasma AST was a good marker of fibrosis and activity, while plasma cholesterol was of interest in the evaluation of activity and vacuolation. Pre-prandial serum bile acids were also positively correlated with vacuolation.

Interestingly, plasma triglycerides were not associated with any histological feature, as could have been predicted based on previous human and animal studies [6, 8, 9, 10, 13,

15]. In the present study, it was found that HCCA developed in vacuolated livers, as 67% (4/6) of the dogs presenting HCCA had a vacuolation grade of G3. However, the number of dogs with HCCA was not sufficient to make significant conclusions, although this statement would be in accordance with previous observations [2].

Biopsies were either surgically made or completed using TruCut® device instead of the fine needle technique because the hepatopathologist was a human pathologist and was used to working on TruCut® biopsies. The TruCut® technique allows deeper sampling compared with fine needle biopsies, which mostly sample the hepatic surface and can lead to misinterpretation of histologic lesions [12]. Having specimens read by a human hepatopathologist can be seen as a limitation, although in the present study, the pathologist had extensive experience in interpreting canine hepatic specimens and had also specialised in human liver histology, which can be even more interesting regarding comparative pathology and the aetiopathogenic hypothesis. This also explains why the authors used the METAVIR scoring system to grade histological lesions. The METAVIR scoring system was initially built for the evaluation of human chronic hepatitis and is routinely used in human liver histology. The authors of the present study carefully read the WSAVA guidelines regarding the evaluation of liver biopsies, and the features of the METAVIR scoring system fit the criteria and recommendations listed by the liver study group [7].

Currently, the aetiology of hepatic vacuolation remains undetermined. In a previous research abstract, the authors of the present study compared the histological criteria of hepatic vacuolation with those of canine hyperadrenocorticism, and similar cellular and mitochondrial modifications were noted, which could be compatible with alterations in the metabolism of glucocorticoids [5]. Mitochondria play an important role in oxidative stress, and a recent study showed that hepatic mitochondrial dysfunction precedes the development of non-alcoholic fatty liver disease (NAFLD) in obese rats [17]. Similarly, humans suffering from non-alcoholic steatohepatitis (NASH) in the advanced fibrosis stage have low circulating di-hydro-epi-androsterone (DHEA) values. Low DHEA is associated with increased susceptibility to hepatocellular oxidative injury, and increased oxidative stress with subsequent mitochondrial dysfunction is a feature of both animal models of steatohepatitis and humans with NAFLD [4]. In the present study, castrated dogs were more likely to have an elevated fibrosis score than non-castrated dogs, which is in agreement with the statement above, given that lower concentrations of circulating DHEA are to be expected in castrated dogs.

One limitation of the present study was the small size of the cohort. More correlations might have been found with a bigger number of dogs, but the contrary could also have been possible. Another limitation was the age of the dogs. Despite a wide range (from 1 to 13 years), the mean age was 8.3 with a median of 8.5, which indicates that the population

studied here was quite old, and this may have influenced the results. The sex distribution was also a potential bias, with a ratio of 1 male to 3 females. Including dogs with HCCA was done purposefully, as the authors wanted to study potential associations between the presence of HCCA and clinical/biochemical/histological features. This inclusion may also have created a bias in the results, especially regarding histological and biochemical data, although there were only 6 dogs with HCCA. Another limitation was the use of the ACTH stimulation test and LDDT instead of systematic LDDT to rule out hyperadrenocorticism before including the dogs that were suspect for this disease. The sensitivity of the ACTH stimulation test is weaker than that of LDDT, and some cases of hyperadrenocorticism may have been misinterpreted and included in the study [14]. Regarding the exclusion of dogs with neoplasia or suspected neoplasia other than HCCA, thoracic radiographs were not performed systematically, and some dogs may have had intra-thoracic masses that were not detected.

## Conclusion

In the present study, Scottish Terriers with hepatic histological lesions such as vacuolation may or may not have clinical signs, which do not seem to be associated with histological analysis regarding activity, fibrosis and vacuolation grades. In the cohort studied here, vacuolation was generally mild to moderate while fibrosis and activity were low for most of the dogs. From a biochemical point of view, plasma ALT activity was positively correlated with all the histological features, which implies that this variable might be a good indicator of what can be expected from the histological results. Plasma AST activity, cholesterol and pre-prandial serum bile acids were also positively correlated with fibrosis/activity, activity/vacuolation and vacuolation alone, respectively. Plasma ALP activity was significantly more elevated in dogs with HCCA. It seems plausible that HCCA develops in moderately to severely vacuolated livers, as 67% of the dogs with HCCA had a vacuolation grade of G3.

## Conflict of interest

The authors have no conflict of interest to declare.

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