Structured summary:

Objectives: Mineral opacities within the liver or biliary system are usually considered incidental but have been reported in one dog to be associated with choledocoliths, and biliary tree mineralisation is seen occasionally in dogs with bile duct carcinoma. The purpose of this study was to assess the prevalence, clinical significance and breed distribution of linear branching mineralisation superimposed on the hepatic silhouette from radiographs of dogs from one referral institution. The hypothesis was that linear branching mineralisation is an incidental finding in dogs.

Methods: Medical records and images of dogs with branching mineralisation seen on radiographs +/- ultrasound in the liver were reviewed retrospectively to characterize and assess their clinical significance.

Results: Only 17 cases were found over 30 years. Out of those, 41% were Cavalier King Charles Spaniels (CKCS), and only 23% of all dogs were diagnosed with a disease known to be affecting the hepatobiliary system primarily or secondarily. The mineralisation had a predominantly ventral distribution in all cases. Five dogs had repeat radiographs, and no change in the pattern of mineralisation was detected in four dogs and only one dog developed the pattern 6 years after being diagnosed with cholangiohepatitis. Serum calcium concentrations were normal in all patients. Liver enzymes were only markedly elevated in the dog which was diagnosed with cholangiohepatitis. Histology performed on 3 patients did not show convincing evidence of primary liver disease or any pathology to cause this pattern of mineralisation.

Impact: This study suggests that branching mineralisation in the liver parenchyma is a rare finding in dogs with little to no clinical significance and that Cavalier King Charles Spaniels may

be predisposed. Therefore, biopsy of the liver of these dogs where there is no clinical or clinicopathological evidence of liver disease might be avoided in these cases.

Key words: radiography, biliary, mineralisation, dog.

Introduction:

Various conditions have been reported to cause mineral opacities within the liver or biliary system in dogs (Lamb 1991). Different patterns of mineral opacification have been described in the hepatic parenchyma: a dense mass involving an entire lesion, a curvilinear ring of calcification or large centrally located coarse mineralisation pattern, or multiple or solitary foci of mineralisation located eccentrically within a complex heterogeneous mass. In humans, these mineral opacities may be associated with chronic liver disease and bile obstruction, however, they are often of no clinical significance and are believed to originate from areas of cellular necrosis (Araujo Bezerra 2003). The most common causes of these clinically significant hepatic mineralisation in humans are neoplasia (primary hepatic or metastatic) or cystic lesions. (Paley and Ros 1998). Parenchymal mineralisation may appear following chronic conditions such as chronic hepatopathy, or parasitic infection (for example, schistosomtosis, histoplasmosis, fascioliasis, echinococcosis) (Grange et al. 1974, Monzawa et al. 1993, Pan et al. 1999, Polat and Atamanalp 2009), bacterial infections (for example, tuberculosis) (Maglinte et al. 1988, Sheen-Chen et al. 2001, Wong and Ng 1993), viral infections (Konen et al. 2000, Saikia et al. 2007) or with diseases causing tissue necrosis such as neoplasia (Bayraktutan et al. 2014, Inoko et al. 2015, Mitsudo et al. 1995, Murakami et al. 2013, Nagakura et al. 1999, Shapiro et al. 1988, Wang et al. 2014), granulomas (Akimoto et al. 1993), hematomas or abscesses (Paley and Ros 1998, Reeder 1975, Stoupis et al. 1998). Another pattern of mineralisation which has been described in

the literature is the linear branching mineralisation pattern which we propose to study in this paper. One paper reports two cases of biliary wall calcification in humans in Langerhans cell histiocytosis (Caruso et al. 2008), detected by multi-detector row CT. In the biliary system, choleliths can be seen as focal mineral opacities in the area of the gallbladder or bile ducts.

Linear branching mineralisation extending peripherally has been described in one dog associated with choledocholiths (Cantwell 1983), and mineralisation of the biliary tree is seen occasionally in dogs with bile duct carcinoma (Thrall 2013).

Branching mineralisation within the hepatic parenchyma is rare and can be seen occasionally in small breed dogs (reported in older Terrier breed dogs (Thrall 2013)). The clinical significance of these findings in dogs remains unknown. A search of the literature revealed no studies focusing on hepatic or biliary mineralisation in the veterinary literature. One report in dogs with Caroli's disease (congenital dilation of the bile ducts) describes biliary mineralisation (Gorlinger et al., 2003). Of the eight dogs included in that study, seven had intra and extra-hepatic bile duct mineralisation on ultrasound examination. All the dogs included in the study had increased serum liver parameters (alkaline phosphatase (ALP) and bile acids).

The purpose of this study was to assess the prevalence, clinical significance and breed distribution of linear branching mineralisation overlying the hepatic silhouette on radiographs in dogs from one referral institution. The hypothesis was that linear branching mineralisation is an incidental finding in dogs.

Materials and Methods

The radiology database of a referral hospital was retrospectively searched for all cases of hepatic and biliary tract mineralisation reported between 1982 and 2015. The primary inclusion criteria was the presence of linear branching mineralisation superimposed on the hepatic silhouette on thoracic or abdominal radiographs, with or without gallbladder involvement. Cases where only the gallbladder was mineralised were excluded from the study, along with cases which showed only a single or multiple clearly identifiable gallbladder stones. Gallbladder stones were defined as round to oval smooth edged mineral opacity structures overlying the liver in the area of the gallbladder on the radiographs. For each case, all the radiographic projections available were reviewed and the distribution of the mineralised pattern was noted. Still ultrasound images were reviewed when present and the reports were checked afterwards in conjunction with the images. Signalment, and clinical history was retrieved. When available, histology and clinical pathology (serum biochemistry and haematology) results were included for each case.

Results

A total of 24 cases were found upon searching the radiology database. Out of those cases, the images and medical records could not be found for 6 cases. These six cases comprised three Cavalier King Charles Spaniels, two crossbreed dogs, and one English springer spaniel. These cases were excluded from the study. One additional dog was excluded as it presented with stippled amorphous mineralisation throughout the entire hepatic parenchyma on the radiographs, unlike the branching pattern described in this study.

In total, 17 dogs met the inclusion criteria. Signalment and associated diseases are shown in Table 1. Mean age was 9.7 years, with a standard deviation of 3.09, and median age was 9.5

years. Of those, 6/17 (35 %) were female neutered, 2/17 (12%) female entire, 7/17 (41%) male neutered and 2/17 (12 %) male entire. A total of 7/17 (41 %) of dogs were Cavalier King Charles Spaniels, 2/17 (12 %) were boxers, 5/17 (29 %) were mix breed, of which one was a German Shepherd dog mix, one a Yorkshire Terrier cross and one a Flat Coated Retriever cross Collie . Finally there were one of each Lhasa Apso, Shih Tzu and a Labrador retriever.

At the time of initial imaging examination, no dog was on any treatment for hepatic disease or had received any steroids. Four dogs (23 %) were diagnosed with a disease known to affect or potentially could affect the hepatobiliary system, whether primarily or secondarily: one dog was definitely diagnosed with hyperadrenocorticism (dog 1); one dog with hepato-cutaneous syndrome (dog 2); one dog with cholangiohepatitis (dog 17) and one dog (dog 7) with pancreatitis (which could be associated with extrahepatic biliary tract obstruction). Additionally, one dog (dog 13) presented with clinical and biochemical signs highly suspicious of hyperadrenocorticism, but the owners declined further testing for the disease, so no definitive diagnosis was made. Two dogs were diagnosed with diseases known to cause ectopic mineralisation (one with parathyroid carcinoma (dog 16) and one (dog 4) with chronic renal failure), but no other areas of mineralisation were found in those patients and total and ionized serum calcium concentrations were within reference range in all these patients.

All the other dogs (10/17) were diagnosed with diseases seemingly unrelated to the branching pattern identified on radiographs, although 6/10 cases had neoplasia elsewhere. Out of those 10 dogs, two did not have accessible biochemistry results (dogs 3 and 15). The final diagnosis in these 10 dogs was maxillary tumour (2 cases: dogs 3 and 6), insulinoma (dog 5), mammary tumour (dog 8), immune mediated polymyositis (dog 9), malignant melanoma (dog 10),

mediastinal mesothelioma (dog 12), laryngeal collapse (dog 13), intervertebral disc disease (dog 14) and lumbosacral stenosis (dog 15).

Repeat radiographs were taken in 5 dogs (dogs 1, 7, 9, 13 and 17). The dog with cholangiohepatitis (dog 17) had initial radiographs which did not show any mineralisation, but repeat radiographs 6 years later showed a branching mineralisation pattern. In the other 4 dogs where repeat radiographs were taken at a later date, the mineralisation appeared the same, whether at 2 months (1 dog), 10 months (1 dog) or 2 years (2 dogs) after initial radiographs, including in one dog treated with ursodeoxycholic acid (Destolit, Norgine Ltd) after the mineralisation were found.

On radiographs; the branching pattern was observed on the right side in all patients in which a dorsoventral projection was obtained (5 cases: dogs 2, 7, 10, 12 and 13), aside from one patient where it was midline with slight left sidedness. On the lateral projections, the mineralisation was mostly located ventrally (level with or ventral to the height of the thoracic portion of the caudal vena cava on the lateral projection). This distribution was seen in all but one patient.

In seven cases where abdominal ultrasonography had been performed (dogs 1, 2, 4, 7, 11, 12 and 16), these lesions appeared as linear hyperechoic areas causing acoustic shadowing in all dogs. Colour flow Doppler examination showed no flow within these structures in any angle or scanning plane. Figure 1 illustrates the imaging findings.

Clinical pathology results were available for 12 patients (5 CKCS and one of each Shih Tzu, boxer, Labrador retriever, Yorkshire cross, Mix breed, Labrador retriever cross and Lhasa Apso). The abnormal results are presented in Table 2. Mild elevations of liver enzymes and/or bile acids were present in three cases; the Shi Tzu (dog 6), the CKCS with pancreatitis (dog 7) and the

CKCS with chronic kidney disease (dog 4). Marked elevations of liver enzyme concentrations were detected in the Labrador retriever cross diagnosed with cholangiohepatitis (dog 17) and the Yorkshire terrier cross with stiffness (dog 11). In all cases, total calcium was normal, aside from the dog with hypertension (dog 4), which had a calcium of 2.96 mmol/l (reference range 2.3-2.8) but a normal ionized calcium (1.37 mmol/l, reference range 1.26-1.5 mmol/l). Haematology was unremarkable in all cases except for mild leucocytosis in the dog with laryngeal collapse (dog 13) which was interpreted as stress reactive leucocytosis.

Liver histology was available in three cases. The first case was the CKCS with hepatocutaneous syndrome (dog 2). This revealed macronodular regeneration with marked bile duct hyperplasia and some fibrosis. Histopathology revealed periportal lymphoplasmacytic inflammation and fibrosis and calcified material within the bile duct, confirming that the calcification was predominantly of the bile in this case. The second case was the dog with cholangiohepatitis (dog 17), which had ultrasound-guided tru-cut biopsies of the liver performed, which showed severe peri-portal fibrosis with a mild lymphocytic infiltration, suggesting chronic cholangiohepatitis, but no calcification.

Finally, the third case was a 10 year old female CKCS (dog 8). This patient had a routine dental done at another practice and radiographs were taken as mild elevation of liver enzymes and bile acids had been found on two occasions when routine laboratory tests were performed a month and three months prior to presentation. This dog also had been previously diagnosed with syringomyelia, degenerative mitral valve disease and vaginitis. After the initial radiographs were taken, the dog was prescribed a 10 day course of antibiotics, and was given S-adenosyl methionine and sylibin. No further investigations for the branching mineralisation were pursued at the time as the animal was not showing any clinical signs and yearly routine biochemistry did

not show any change in the hepatic enzymes. Two years later, the dog developed a pyometra. During surgery for ovariohysterectomy, the liver was macroscopically examined and determined to be normal, and biopsies of the liver were taken and sent out for histopathology. At the time of biopsies, radiographs were retaken, and the linear branching mineralisation previously described in this patient appeared stable on radiographs. The bile acids started to decrease after the ovariohysterectomy. On histopathology, the hepatic acinar architecture was maintained, the sinusoids contained increased neutrophils. Portal and peri-acinar areas also contained some segmented leukocytes, many of which were immature neutrophils. Perinuclear hepatocytes and Kupffer cells contained an increased amount of cytoplasmic pigment, but no bile canalicular plugging was seen. No fibrosis or necrosis were noted. The final histopathological diagnosis was sinusoidal leucocytosis with mild acute reactive hepatitis which was attributed to the concurrent pyometra. No convincing evidence of primary liver disease or calcification was seen in the samples submitted.

Discussion

In this report, branching hepatic mineralisation is described in a group of dogs for the first time in the veterinary literature. The prevalence of branching hepatic mineralisation on radiographs in dogs appears to be very low, as only 24 cases were found on a search spanning over 30 years in a single referral centre. Cavalier King Charles Spaniels were over-represented in this study, accounting for 41 % of cases included in the study, whereas over this time period, only 3.2% of dogs seen at the hospital and 4.1 % having radiographs of the liver were Cavalier King Charles Spaniels. In this study, 3/7 Cavalier King Charles Spaniels (i.e. 47 %) had a final diagnosis of a disease which was likely to be related to the hepato-biliary system, of which 1/7 (14%) was a primary hepatic disease and 2/7 (28%). were secondary diseases known to affect hepatic function,

but did not show any clinical evidence of hepatic disease. In the general population, primary histopathological hepatic lesions in CKCS have been shown to be found in 11 % of cases showing no ante-mortem signs of hepatic disease (Kent et al. 2016), which is similar to the results found in this study. In other dog breeds in this study one out of ten cases (10%) showed evidence of primary hepatic involvement.

The branching mineralisation did not appear to be causing any clinical signs in 10/17 (58%) of cases in this study and 8/10 of those dogs had biochemistry data available. Only four dogs were diagnosed with chronic diseases related to the hepatobiliary system and one additional dog had suspected but not confirmed hyperadrenocorticism. Two dogs were diagnosed with diseases which could potentially account for ectopic mineralisation (chronic renal disease and parathyroid carcinoma). However, none of these dogs had elevated total or ionized calcium concentrations at the time they were measured. It was possible that calcium elevations were transient in these dogs. However, this would not explain the very low prevalence of the branching pattern seen in this study compared to the much higher prevalence of renal disease and parathyroid carcinoma in animals, nor the breed predisposition towards Cavalier King Charles Spaniels. It is therefore unlikely that the mineralisation found in the liver of these dogs was due to the patient's underlying disease.

It is not possible to know whether these mineralisation occurred due to previous hepatic injury which would have gone unnoticed, or whether these mineralisation is incidental. Primary liver disease in the form of chronic cholangiohepatitis was diagnosed in only one dog and the linear branching hepatic mineralisation was found on radiographs 6 years after the condition had been successfully treated. Another CKCS with biliary mineralisation also had choleliths and pancreatitis. Additionally, in four cases, repeat radiographs showed no change in the amount or

pattern of mineralisation up to two years after the initial finding. This suggests that in the majority of dogs, branching mineralisation in the liver is an incidental finding. However, there appears to be a breed predisposition in the Cavalier King Charles Spaniels, which is also the breed in which an underlying hepatic disease was the most diagnosed. The cause of the mineralisation therefore remains unknown but it is possible that Cavalier King Charles Spaniels have a predisposition to mineralisation of the biliary tract which could be associated with exposed to irritants such as choleliths and inflammation and potentially also subclinical exposure to bacteria and other antigens from the gut. This hypothesis would need further work to confirm it. There is an association between biliary calcification and parasite infections in humans and in veterinary patients (Scharf et al. 2004)and it would also be interesting to know if the dogs in this study had intestinal parasites, but the retrospective nature of the study precluded this.

Histopathology of the liver was performed in three patients in this study and showed that only the case with cholangiohepatitis had evidence of chronic liver disease. Although this constitutes a small number of patients, this finding associated with the low number of patients with a disease related to the liver which have been found in this study suggests that hepatic biopsy of dogs with these lesions identified on radiographs but with no clinical or clinicopathological evidence of liver disease is not indicated, as the clinical significance of these lesions appears low.

There are limitations to this study. Firstly, it was a retrospective study so full details were not available for all cases. In addition, there was only a small number of cases matching the inclusion criteria. However, branching mineralisation in the liver has a very low prevalence. Furthermore, histopathology was only available on three cases, and clinical pathology results were only available in 12 patients. Nonetheless, this study has the largest number of cases of biliary mineralisation reported so far.

Conclusions:

Hepatic linear branching mineralisation is uncommon in dogs. Cavalier King Charles Spaniels appear to have an increased prevalence. Many dogs in which hepatic mineralisation were seen had a final diagnosis which was unrelated to any hepatic disease, and only one dog was seen to have developed this pattern following a long period of chronic hepatic disease, suggesting that this is likely to be an incidental finding, and that systematic liver biopsies are not indicated to pursue this finding.

Conflict of interest: No conflicts of interest have been declared

References:

Akimoto, M., Yoshikawa, J., Matsui, O., et al. 1993. Hepatic granuloma with progressive calcification: CT appearance. *Radiat Med,* 11**,** 170-2.

Araujo Bezerra A.S, D. I. G., Martelli P, Pinto G, et al. 2003. Liver calcifications: frequency and significance. *Radiol Bras,* 36**,** 199-205.

Bayraktutan, U., Ogul, H., Kantarci, M., et al. 2014. Education and imaging. Hepatobiliary and pancreatic: calcified perihepatic and capsular metastases. *J Gastroenterol Hepatol,* 29**,** 664.

Cantwell HD, B. W., Hanika-Rebar C, et al. 1983. Radio-opaque hepatic and lobar duct choleliths in a dog. *Am Anim Hosp Assoc,* 19**,** 373.

Caruso, S., Miraglia, R., Maruzzelli, L., et al. 2008. Biliary wall calcification in Langerhans cell histiocytosis: report of two cases. *Pediatr Radiol,* 38**,** 791-4.

Gorlinger, S., Rothuizen, J., Bunch, S., et al. 2003. Congenital dilatation of the bile ducts (Caroli's disease) in young dogs. *J Vet Intern Med,* 17**,** 28-32.

Grange, D., Dhumeaux, D., Couzineau, P., et al. 1974. Hepatic calcification due to Fasciola gigantica. *Arch Surg,* 108**,** 113-5.

Inoko, K., Tsuchikawa, T., Noji, T., et al. 2015. Hilar cholangiocarcinoma with intratumoral calcification: A case report. *World J Gastroenterol,* 21**,** 10926-30.

263 Kent, A. C., Constantino-Casas, F., Rusbridge, C., et al. 2016. Prevalence of pancreatic, hepatic
264 and renal microscopic lesions in post-mortem samples from Cavalier King Charles
265 spaniels. *J Small Anim Pract,* 57**,** 188-93.
266 Konen, O., Rathaus, V., Bauer, S., et al. 2000. Progressive liver calcifications in neonatal
267 coxsackievirus infection. *Pediatr Radiol,* 30**,** 343-5.
268 Lamb CR., K. L., McMillan MC. 1991. Diagnosis of calcification on abdominal radiographs.
269 *Veterinary Radiology and Ultrasound,* 32**,** 211-220.
270 Maglinte, D. D., Alvarez, S. Z., Ng, A. C., et al. 1988. Patterns of calcifications and
271 cholangiographic findings in hepatobiliary tuberculosis. *Gastrointest Radiol,* 13**,** 331-5.
272 Mitsudo, K., Watanabe, Y., Saga, T., et al. 1995. Nonenhanced hepatic cavernous hemangioma
273 with multiple calcifications: CT and pathologic correlation. *Abdom Imaging,* 20**,** 459-61.
274 Monzawa, S., Uchiyama, G., Ohtomo, K., et al. 1993. Schistosomiasis japonica of the liver:
275 contrast-enhanced CT findings in 113 patients. *AJR Am J Roentgenol,* 161**,** 323-7.
276 Murakami, T., Morioka, D., Takakura, H., et al. 2013. Small hepatocellular carcinoma with ring
277 calcification: a case report and literature review. *World J Gastroenterol,* 19**,** 129-32.
278 Nagakura, S., Shirai, Y., Yamai, K., et al. 1999. Calcification in mucinous cholangiocellular
279 carcinoma. *Hepatogastroenterology,* 46**,** 465-6.
280 Paley, M. R. & Ros, P. R. 1998. Hepatic calcification. *Radiol Clin North Am,* 36**,** 391-8.
281 Pan, K. T., Hung, C. F., Tseng, J. H., et al. 1999. Hepatic calcification by sequelae of chronic
282 schistosomiasis japonica: report of four cases. *Changgeng Yi Xue Za Zhi,* 22**,** 265-70.
283 Polat, P. & Atamanalp, S. S. 2009. Hepatic hydatid disease: radiographics findings. *Eurasian J*
284 *Med,* 41**,** 49-55.
285 Reeder, M. M. 1975. Liver calcification. *Semin Roentgenol,* 10**,** 171.

Saikia, N., Talukdar, R., Singal, D. K., et al. 2007. Hepatic calcification following dengue virus-induced fulminant hepatic failure. *Indian J Gastroenterol,* 26**,** 90-2.

Scharf, G., Deplazes, P., Kaser-Hotz, B., et al. 2004. Radiographic, ultrasonographic, and computed tomographic appearance of alveolar echinococcosis in dogs. *Vet Radiol Ultrasound,* 45**,** 411-8.

Shapiro, R. S., Mendelson, D. S., Norton, K. I., et al. 1988. Case report: calcified liver metastases from osteosarcoma. *J Comput Tomogr,* 12**,** 196-8.

Sheen-Chen, S. M., Chen, M. C., Hu, T. H., et al. 2001. Computed tomography and angiography in hepatic tuberculosis mimicking liver tumor. *Int J Tuberc Lung Dis,* 5**,** 876-8.

Stoupis, C., Taylor, H. M., Paley, M. R., et al. 1998. The Rocky liver: radiologic-pathologic correlation of calcified hepatic masses. *Radiographics,* 18**,** 675-85; quiz 726.

Thrall 2013. The Liver and Spleen. In: Textbook of Veterinary Diangostic Radiology. 6th edn, St. Louis, Elsevier Saunders. pp 684

Wang, C. E., Sun, C. J., Huang, S., et al. 2014. Extensive calcifications in portal venous system in a patient with hepatocarcinoma. *World J Gastroenterol,* 20**,** 16377-80.

Wong, M. K. & Ng, S. E. 1993. Computed tomographic findings of abdominal tuberculosis--report of five cases. *Ann Acad Med Singapore,* 22**,** 802-6.

Figure and table legends:

Table 1. Signalment and diagnosis in 17 dogs with branching mineralisation pattern on radiographs. F, M: female/male, N: neutered, E: Entire. CKCS: cavalier King Charles spaniel, GSDx= German Shepherd mix. AUS: abdominal ultrasound, TXR: thoracic radiographs, AXR: abdominal radiographs, echo : echocardiography. Case numbers are on the left of the table.

Table 2. Biochemistry parameters in 5 of 12 dogs with linear branching mineral opacities in the liver where one or more of the liver parameters were abnormal. Reference ranges in parentheses

Figure 1: Left lateral thoracic radiographs showing branching mineralisation pattern overlying the hepatic silhouette in two dogs (A: Dog 5, B: Dog 6)

Figure 2: Ultrasound image of the liver showing hyperechoic structures within the bile ducts/parenchyma of two dogs, corresponding to the branching mineralisation seen on ultrasound: dog 11 (A and B), dog 5 (C), White arrows point to the mineralised areas.