

This is the peer reviewed version of the following article:

Jones, I. D., Lamb, C. R., Drees, R., Priestnall, S. L. and Mantis, P. (2016), ASSOCIATIONS BETWEEN DUAL-PHASE COMPUTED TOMOGRAPHY FEATURES AND HISTOPATHOLOGIC DIAGNOSES IN 52 DOGS WITH HEPATIC OR SPLENIC MASSES. *Veterinary Radiology & Ultrasound*, 57: 144–153. doi: 10.1111/vru.12336

which has been published in final form at <http://dx.doi.org/10.1111/vru.12336>.

This article may be used for non-commercial purposes in accordance with [Wiley Terms and Conditions for Self-Archiving](#).

The full details of the published version of the article are as follows:

TITLE: Associations between dual-phase computed tomography features and histopathologic diagnoses in 52 dogs with hepatic or splenic masses

AUTHORS: Jones, I. D., Lamb, C. R., Drees, R., Priestnall, S. L. and Mantis, P.

JOURNAL TITLE: *Veterinary Radiology & Ultrasound*

VOLUME/EDITION: 57/2

PUBLISHER: Wiley

PUBLICATION DATE: March 2016

DOI: 10.1111/vru.12336

1 **Associations between dual-phase CT features and histopathologic diagnoses in 52 dogs with**
2 **hepatic or splenic masses**

3

4 I.D. Jones, C.R. Lamb, R. Drees, S.L. Priestnall, P. Mantis

5 Department of Clinical Sciences and Services (Jones, Lamb, Drees, Mantis) and Department of
6 Pathology and Pathogen Biology (Priestnall), The Royal Veterinary College, Hawkshead Lane,
7 North Mymms, Hertfordshire AL9 7TA, UK.

8

9 Key words: Computed tomography, dog, hepatic disease, neoplasia, splenic disease

10 Running head: Dual-phase CT of hepatic and splenic masses

11

12 Funding Sources: unfunded

13

14 Presented at the EVDI Annual Conference, Utrecht, The Netherlands, 27-29th August 2014.

15 **Abstract**

16 Ability to non-invasively differentiate malignant from non-malignant abdominal masses would
17 aid clinical decision-making. The aim of this retrospective, cross-sectional study was to identify
18 features in dual-phase computed tomographic (CT) studies that could be used to distinguish
19 malignant from non-malignant hepatic and splenic masses in dogs. Medical records were
20 searched for dogs that had an abdominal dual-phase CT examination, a hepatic or splenic mass,
21 and subsequent histopathologic diagnosis. CT images were acquired prior to and <30s (early
22 phase) and >60s (delayed phase) after intravenous contrast administration. Fifty-two dogs with
23 55 masses were studied: 24 hepatic, including 14 (58%) malignant and 10 (42%) non-malignant;
24 31 splenic, including 18 (58%) malignant and 13 (42%) non-malignant. There was substantial
25 overlap in the pre- and post-contrast CT features of malignant and non-malignant hepatic and
26 splenic masses. Regardless of histologic diagnosis, hepatic masses most frequently showed
27 marked, generalized enhancement in early phase images that persisted in the delayed phase.
28 Splenic hemangiosarcoma and nodular hyperplastic lesions most frequently showed marked,
29 generalized enhancement in early phase images that persisted in delayed images whereas most
30 splenic hematomas had slight enhancement in early phase images. All splenic hematomas and
31 77% of the hemangiosarcomas had contrast accumulation compatible with active hemorrhage.
32 There were no other significant differences in quantitative or categorical CT data between
33 malignant and non-malignant hepatic or splenic masses. Dual-phase CT of dogs with hepatic or
34 splenic masses provides limited specific diagnostic information.

35 **Introduction**

36 Hepatic and splenic masses are encountered frequently in dogs and reflect a range of
37 pathological conditions including malignant neoplasia such as hepatocellular carcinoma and
38 hemangiosarcoma, benign neoplasia such as hepatocellular adenoma and non-neoplastic
39 conditions such as hepatic nodular hyperplasia and hematoma.¹⁻⁶ Optimal management depends
40 on accurate diagnosis and staging.⁷ Ability to non-invasively differentiate malignant from non-
41 malignant masses would aid clinical decision-making.

42 In humans with a suspected hepatic mass, multi-phase computed tomographic (CT) protocols
43 have been used to examine the pattern of enhancement of hepatic lesions following
44 administration of intravenous contrast medium.^{8,9} The purpose of multi-phase CT protocols is to
45 reveal differences in the vascular supply to lesions that reflect features of diagnostic significance.
46 For example, hypervascular hepatic lesions (such as neoplasms with marked neovascularization)
47 derive their blood supply primarily from the hepatic artery, so show relatively marked contrast
48 uptake in early post-contrast CT images.⁸⁻¹⁰ Hypovascular lesions (such as metastases with
49 necrosis) generally show reduced contrast uptake compared to the surrounding liver in late post-
50 contrast CT images.^{8,9} However, differentiation of malignant and non-malignant hepatic lesions
51 using contrast uptake characteristics is complicated because non-malignant lesions (such as
52 hepatic nodular hyperplasia) may also show signs of early marked contrast uptake followed by
53 reduced uptake in later images, which mimics the appearance of malignant neoplasms.⁸

54 In conscious humans, peak opacification of the hepatic arteries occurs at 15-25s, peak
55 opacification of the portal veins occurs at 45-55s, and peak opacification of the hepatic veins
56 occurs at 60-70s after the start of contrast medium injection.^{8,11} Triple-phase CT protocols,
57 involving images acquired during the early and late hepatic arterial phase and the portal phase

58 were studied because of the potential for increased accuracy of diagnosis^{9,12,13}, but it was found
59 that acquiring two arterial phases carried no significant advantage.^{14,15} Triple-phase CT protocols
60 are now used less frequently in human clinical practice than a dual-phase approach involving an
61 hepatic artery-dominant phase (HAP) and a portal venous-dominant phase (PVP) with CT
62 images acquired using time delays of 30-35s and 60-65s after the start of intravenous injection,
63 respectively.^{8,14-17} In dual-phase CT protocols to examine the liver, images are acquired after
64 peak vessel opacification because contrast accumulation in parenchymal lesions occurs after
65 peak vessel opacification.^{8,11,14} Additional delayed phase images at 180s are recommended by
66 some authors¹⁸ but are considered low-yield by others.¹⁹ In general, HAP images aid detection
67 of hypervascular hepatic lesions^{8,12-16} whereas PVP images and delayed images help characterize
68 certain hepatic lesions, such as hemangiomas, and aid detection of active hemorrhage.²⁰

69 Dogs with hepatic masses have not been investigated using dual-phase CT. Instead, triple-phase
70 CT studies have been described, with images acquired at 13-20s (HAP), 30-40s (early PVP), and
71 120s (equilibrium or delayed phase) following contrast administration.^{21,22} These studies found
72 evidence of associations between the pattern and degree of post-contrast enhancement and the
73 pathologic diagnosis of hepatic masses, although there was substantial overlap.^{21,22} Most
74 hepatocellular carcinomas showed heterogeneous enhancement in HAP, PVP and delayed phase
75 images, often had a central zone of hypoattenuation, and were hypoattenuating compared to the
76 liver in portal and delayed phase images.^{21,22} Hepatic adenomas were more likely to show diffuse
77 enhancement in HAP images and to retain contrast in PVP images.²¹ Hepatic hyperplastic
78 nodules were relatively homogeneous, frequently showed marked enhancement in HAP and PVP
79 images, and isoattenuation compared to adjacent normal liver in delayed phase images.^{21,22} In

80 one study, hepatic metastases consistently appeared homogenous with hypoattenuation compared
81 to the liver in all post-contrast CT images.²²

82 Multi-phase CT imaging of canine splenic masses has not been reported. A single-phase CT
83 study of splenic masses in dogs used images acquired prior to and approximately 60s after
84 intravenous contrast administration.²³ It was found that malignant lesions, mainly
85 hemangiosarcoma, had lower attenuation than non-malignant masses in pre-contrast images and
86 enhanced less than surrounding splenic parenchyma in post-contrast images.²³

87 The aim of the present study was to review post-contrast CT images acquired using a dual-phase
88 protocol in a series of dogs with histopathologically-confirmed hepatic or splenic masses in order
89 to identify features that could be used to distinguish malignant from non-malignant masses. The
90 term non-malignant is used here to encompass benign neoplasms and non-neoplastic mass
91 lesions.

92

93 **Materials and Methods**

94 Records of the Queen Mother Hospital for Animals of The Royal Veterinary College in the
95 period 2008-2014 were reviewed in a retrospective, cross-sectional study. Inclusion criteria were
96 dogs that had a dual-phase, post-contrast CT study of the abdomen and subsequent
97 histopathologic diagnosis of an hepatic or splenic mass. For the purposes of this study, mass was
98 defined as a focal parenchymal lesion with displacement of adjacent structures and >10mm
99 maximal transverse dimension. Dogs in which the CT examination and histological diagnosis
100 were separated by 30 days or more were not included. Dogs in which diagnosis was based on
101 cytology were not included.

102 CT scans were routinely performed in anaesthetized or sedated patients in sternal recumbency.
103 All scans were obtained using a 16-slice MDCT scanner (MX 8000 IDT, Philips Medical
104 Systems, Cleveland, USA). CT settings were helical acquisition, slice thickness 3mm, image
105 reconstruction interval 1.5mm, helical pitch 0.688, tube rotation time 0.75s, x-ray tube current
106 150 mAs, x-ray tube potential 120kVp, field of view 400mm, matrix 512x512, and medium
107 frequency ('soft tissue') reconstruction algorithm. Scans were performed in a cranial to caudal
108 direction starting at the cardiac apex and terminating at the mid-femoral diaphysis.

109 Post-contrast CT scanning followed a dual-phase protocol introduced at our institution in 2008
110 for all dogs having abdominal CT examinations, except those with suspected portosystemic
111 shunting. In each dog, 2ml/kg of iohexol 300mg/ml (Omnipaque 300, GE Healthcare, Oslo,
112 Norway) was injected intravenously at 2ml/s (maximum pressure 150 psi) using a power injector
113 (Stellant, Medrad Inc., Pennsylvania, USA) via a catheter in a cephalic or saphenous vein.
114 Images of the whole abdomen were acquired prior to, within 30 seconds (early phase) and at
115 least 60 seconds (delayed phase) following the start of intravenous injection of contrast, as
116 described for dual-phase CT protocols for humans.^{8,14-17} . The variable need for entry of the
117 anesthetist into the CT room to examine their patient after the first post-contrast CT acquisition
118 contributed to variations in the timing of the second post-contrast acquisition, which was
119 initiated only after all personnel had exited the CT room.

120 CT images were reviewed by a Board-certified radiologist (PM) who was blinded to the final
121 diagnosis. The reviewer evaluated only transverse images. Where multiple CT examinations
122 were available for a dog, only the first examination was reviewed because later studies were
123 generally obtained after biopsy of the mass, surgery or other treatment. Dogs were excluded if
124 review of their CT images found multiple hepatic or splenic lesions with differing imaging

125 features. This was considered necessary in order to be able to relate the biopsy result in each dog
126 with the corresponding features in CT images.

127 The following patient information was recorded: Site of mass (hepatic/splenic), diagnosis, age at
128 time of study, breed, sex and weight. The catheter site used for contrast administration was also
129 recorded.

130 Commercially available software (OsiriX, Pixmeo, SARL, 266, Rue de Bernex, CH-1233,
131 Bernex, Switzerland) was used for image review and analysis. Window width was 400
132 Hounsfield units (HU), window level was 60 HU. Aortic and portal vein attenuation in early and
133 delayed phase images was recorded. The maximal transverse dimension of each mass was
134 measured using electronic calipers. Normal attenuation value of hepatic/splenic parenchyma was
135 determined based on the mean of three 20mm² regions of interest (ROIs). Attenuation of masses
136 was measured using the maximum circular ROI that could be fitted to each mass. Identical ROIs
137 were used for measurements in pre-contrast images and early and delayed phase images. In pre-
138 contrast CT images, hyperattenuation was defined as at least 10HU greater than normal
139 parenchyma; hypoattenuation was defined as at least 10HU less than normal parenchyma;
140 isoattenuation was defined as attenuation within 10 HU of normal parenchyma.²²

141 The following quantitative variables were recorded: maximal transverse dimension of the mass
142 (mm), mean hepatic/splenic parenchymal attenuation pre-contrast (HU), attenuation of lesion
143 pre-contrast (HU), relative attenuation of lesion (lesion attenuation – mean hepatic/splenic
144 parenchymal attenuation), attenuation of lesion in early phase (HU), early enhancement of lesion
145 (attenuation of lesion in early phase – attenuation of lesion pre-contrast), delayed attenuation of
146 lesion (HU), delayed enhancement of lesion (delayed attenuation of lesion – pre-contrast
147 attenuation of lesion) and contrast accumulation within the lesion (delayed enhancement of

148 lesion – early enhancement of lesion). Slight enhancement was defined as $<10\text{HU}$; marked
149 enhancement was defined as $\geq 10\text{HU}$.

150 The following subjective categorical variables were recorded: appearance of the margin of mass
151 (distinct/indistinct), parenchymal homogeneity of mass in pre-contrast images
152 (homogenous/heterogeneous), hepatic or splenic capsular distortion as a result of mass effect
153 (yes/no), pattern of contrast enhancement (generalized /peripheral), presence of peritoneal fluid
154 (yes/no), mineralization within mass (yes/no), and presence of abnormal hepatic and/or splenic
155 lymph nodes (yes/no).^{22,24}

156 Tissue for histological analysis was acquired by ultrasound-guided tissue core biopsy using 14G
157 or 18G needles, wedge resection at exploratory laparotomy, partial hepatectomy or splenectomy.
158 All histopathologic specimens were reviewed by a Board-certified pathologist (SLP).

159 Normality of continuous data was assessed by visual inspection and the Kolmogorov-Smirnov
160 test. Data did not conform to a Normal distribution, hence differences were tested using a Mann-
161 Whitney U-test. Likelihood ratios (LR) and their 95% confidence intervals (95% CI) were
162 calculated to describe the strength of associations between categorical features and malignancy.
163 Statistical tests were performed using commercially available (SPSS version 16, SPSS Inc.,
164 Chicago, Illinois, USA) and online software (Stats calculator, Centre for Evidence Based
165 Medicine, Toronto, Canada, <http://ktclearinghouse.ca/cebm/practise/ca/calculators/statscalc>).

166

167 **Results**

168 Fifty-two dogs with 55 masses were included: 24 had an hepatic mass, 31 had a splenic mass,
169 and 3 dogs had both a hepatic and splenic mass (2 dogs had primary splenic hemangiosarcoma

170 with metastasis to the liver, and one dog had primary splenic spindle cell sarcoma with
171 metastasis to the liver). Dogs were 11 mixed breed, 7 Labrador retrievers, 5 German shepherd
172 dogs, 3 Flat-coated retrievers, 2 Boxers, 2 Golden retrievers, 2 Schnauzers and 2 West highland
173 white terriers and 1 each of the following breeds: Beagle, Bernese mountain dog, Border collie,
174 Border terrier, Bulldog, Cairn terrier, Chow Chow, Cocker spaniel, Dachshund, Dog-de-
175 Bordeaux, English springer spaniel, Irish setter, Lhasa Apso, Lurcher, Papillon, Rhodesian
176 ridgeback, Tibetan terrier, and Yorkshire terrier. Thirty-two dogs were male (20 neutered) and 20
177 were female (19 neutered). Their median age was 10 years (range 3 – 15 years).

178 Median patient weight was 24kg (4-54kg). Cephalic catheters were used to administer contrast in
179 49 (94%) dogs and saphenous catheters were used in 3 (6%) dogs. In every dog, acquisition of
180 early phase images occurred less than 30s after the administration of contrast. The median delay
181 between contrast administration and delayed phase image acquisition was 120s (range 62-465s).

182 Median aortic attenuation was 207HU (range 110-607HU) in early phase images and 130HU
183 (range 49-219HU) in delayed phase images. Median portal attenuation was 155HU (range 48-
184 315HU) in early phase images and 129HU (range 49-226HU) in delayed phase images.

185 Of the 24 hepatic masses, 14 (58%) were malignant (9 carcinoma, 3 metastatic splenic
186 hemangiosarcomas, 1 metastatic splenic spindle cell sarcoma, 1 hemangiosarcoma) and 10
187 (42%) were non-malignant (6 adenoma, 3 nodular hyperplasia, 1 bile duct hyperplasia). Of the
188 31 splenic masses, 18 (58%) were malignant (13 hemangiosarcomas, 3 sarcomas, 1 histiocytic
189 sarcoma, 1 metastatic renal neuroblastoma) and 13 (42%) were non-malignant (8 hematomas, 5
190 nodular hyperplasia).

191 There were no significant differences in quantitative or categorical CT image data between
192 malignant and non-malignant masses in the liver (Tables 1 & 2) or spleen (Tables 3 & 4).

193 Similarly, there was substantial overlap in the pre- and post-contrast imaging features of the most
194 prevalent types of hepatic and splenic masses (Tables 5 & 6, respectively). Recognizing that the
195 wide variation in vascular attenuation observed in this study could potentially obscure
196 differences between types of hepatic and splenic masses, the analyses were repeated after
197 removing data for 8 dogs with delayed phase images >240s after administration of contrast and
198 the 3 dogs with saphenous catheters; however, the significance of results of statistical tests were
199 unchanged, hence these dogs were retained.

200 Hepatic carcinoma, hepatic nodular hyperplasia and hepatic adenoma most frequently had lower
201 attenuation and were heterogeneous compared to surrounding hepatic parenchyma in pre-contrast
202 images, and the majority of these lesions showed marked, generalized enhancement in the early
203 phase images that persisted in the delayed phase (Figure 1). The majority of hepatic masses
204 lacked contrast accumulation. Splenic hemangiosarcoma, hematoma, and nodular hyperplastic
205 masses were mostly slightly heterogeneous with mean attenuation similar to adjacent splenic
206 parenchyma in pre-contrast images. In early and delayed phase images, splenic
207 hemangiosarcoma and nodular hyperplastic lesions most frequently showed marked, diffuse
208 enhancement in early phase images that persisted in delayed images. Most splenic hematomas
209 had slight enhancement in early phase images but marked enhancement in delayed images, as a
210 result of contrast accumulation (Figure 2).

211

212 **Discussion**

213 In the present study, no CT features were found to be significantly associated with malignant or
214 non-malignant hepatic masses. The most prevalent of the hepatic masses studied – hepatic
215 carcinoma, adenoma, and nodular hyperplasia – each had a similar range of CT features. Most

216 frequently these masses were heterogeneous compared to surrounding hepatic parenchyma in
217 pre-contrast images, and the majority of these lesions showed marked, generalized enhancement
218 in the early phase images that persisted in the delayed phase.

219 The present study employed a dual-phase CT protocol comparable to dual-phase CT protocols
220 used for assessment of hepatic lesions in humans.^{8,14-17} There are no other reports of veterinary
221 studies that used the same dual-phase CT protocol for assessment of hepatic or splenic masses.
222 Two recent studies employed triple-phase CT protocols with images acquired at 13-20s (HAP),
223 30-40s (early PVP) and 120s (delayed phase) following contrast administration.^{21,22} Compared
224 to these, the early phase in the dual-phase CT protocol is similar to the early PVP, and the
225 delayed phase is similar to the delayed phase of triple-phase CT. Omission of HAP images is
226 unlikely to significantly reduce ability to distinguish types of hepatic mass because in previous
227 series a large proportion of masses had similar degrees of post-contrast enhancement in HAP and
228 PVP images.^{21,22}

229 In HAP images, enhancement of tissues represents contrast medium within the intravascular
230 space and the degree of enhancement is determined primarily by the hepatic arterial blood
231 flow.¹⁰ Subsequent tissue enhancement represents additional delivery of contrast via the portal
232 vessels and passage of contrast material from the intravascular to the extravascular space across
233 the capillary basement membrane, hence the degree of tissue enhancement also depends on blood
234 volume and permeability of capillaries.¹⁰ Based on previous studies in humans¹⁶ and dogs^{21,22},
235 hepatic masses containing relatively well-differentiated hepatocytes tend to enhance most
236 strongly in HAP images, probably reflecting both their relatively abundant arterial blood flow
237 and lack of necrotic or hemorrhagic (vascular) components. However, these observations do not
238 represent differences that enable different histologic types of hepatic masses to be distinguished

239 clinically because – as observed in the present study – marked enhancement in early phase
240 images occurs with malignant neoplasia, such as hepatocellular carcinoma, and with non-
241 malignant lesions, such as hepatic adenoma and nodular hyperplasia.^{21,22}

242 Differentiation of hepatocellular carcinoma from hepatic adenoma and hepatic nodular
243 hyperplasia depends on histopathology.^{25,26} Hepatic carcinomas contain pleomorphic hepatocytes
244 arranged in irregular trabeculae separated by loose vascular (sinusoidal) spaces and a variable
245 amount of fibrous connective tissue, and exhibit varying degrees of internal hemorrhage and
246 necrosis. Sinusoidal invasion beyond a fibrous capsule or pseudocapsule and/or intrahepatic
247 metastasis are the key histologic criteria of hepatocellular carcinoma, but the former may be
248 subtle and/or focal in a well-differentiated tumor. Hepatic adenomas contain well-differentiated
249 hepatocytes in rough cords and near normal architecture, but lack portal triads or central veins.
250 Hepatic hyperplastic nodules contain well-differentiated hepatocytes with slightly reduced
251 numbers of portal triads or central veins compared to normal liver, but with a distorted lobular
252 architecture and frequent vacuolar changes representing glycogen or lipid accumulation. It is
253 important to note that the histologic diagnosis of these hepatic lesions depends on cellular and
254 architectural features that exist in a spectrum of severity in which the boundaries between well-
255 differentiated hepatocellular carcinoma and adenoma, and between adenoma and nodular
256 hyperplasia, are not always clearly defined.^{25,26} Consequently, there will be a limit to the degree
257 that CT features, which primarily represent non-specific gross pathologic features, such as
258 pseudoencapsulation, hemorrhage and necrosis²⁷, can be used to deduce the histologic diagnosis
259 of hepatic masses. The lack of significant differences in CT features associated with malignant or
260 non-malignant hepatic masses observed in the present study likely reflects this limitation.

261 Splenic hemangiosarcoma, hematoma, and nodular hyperplastic masses also exhibited variable
262 CT features. In pre-contrast images, most splenic masses were slightly heterogeneous, with
263 median attenuation similar to adjacent parenchyma. In early and delayed phase images, splenic
264 hemangiosarcoma and nodular hyperplastic lesions most frequently showed marked, generalized
265 enhancement in early phase images that persisted in delayed phase images. These results do not
266 corroborate those of a previous study in which malignancy of a splenic mass was associated with
267 hypoattenuation in pre-contrast images and minimal contrast accumulation in post-contrast
268 images, with 55HU in post-contrast images representing a threshold value to distinguish
269 malignant (<55HU) from non-malignant (>55HU) masses.²³ In the present study, there was no
270 difference in median enhancement in delayed phase images between malignant and non-
271 malignant splenic masses with both types of mass having a wide range of post-contrast
272 attenuation values that spanned 55HU.

273 Most splenic hematomas had slight enhancement in early phase images, and all hematomas had
274 contrast accumulation in delayed phase images. This feature could represent active hemorrhage
275 occurring during the CT examination²⁰, but was not unique to hematomas as it was also observed
276 in the majority of hemangiosarcomas. Marked focal contrast accumulation in hemangiosarcomas
277 was reported recently, possibly representing contrast medium in wide vascular spaces within
278 these tumors.²⁸ Splenic malignant neoplasms, most of which are hemangiosarcomas, frequently
279 contain extensive hemorrhage or necrosis and so may be difficult to distinguish from hematoma
280 on the basis of either gross or histologic examination.^{3,29-31} As noted above with respect to
281 hepatic masses, other imaging techniques that depict vascularity of tissues also have limited
282 potential to distinguish the histologic types of splenic mass.³²⁻³⁵

283 The present study is subject to patient selection bias because of the requirement for histologic
284 diagnosis, which necessitated biopsy. Lesions suspected to be malignant were more likely to be
285 considered priorities for biopsy than lesions suspected to be benign (e.g. because they appeared
286 unrelated to clinical signs or were known to be static), hence benign masses may be
287 underrepresented compared to the overall number of masses encountered in the clinical setting.
288 Similarly, hepatic or splenic masses that are routinely diagnosed in our hospital on the basis of
289 cytologic and flow cytometric findings, primarily round cell neoplasms, were not included.

290 Patient factors, the method of contrast injection, and timing of CT image acquisition affect the
291 magnitude of enhancement of tissues observed following intravenous contrast administration.^{11,17}
292 Patient body weight and cardiac output will affect the time of the peak plasma concentration of
293 contrast medium even if the iodide concentration of the contrast agent, its dose and rate of
294 injection all remain constant.^{11,17} Injection duration is the most important injection factor to be
295 considered for determining CT scan timing because it directly affects the time to peak contrast
296 enhancement in an organ or vessel.^{11,17} With an increased duration of contrast injection, the time
297 to peak opacification increases. For example, the protocol used in the present study with an
298 injection rate 2ml/s will result in a slightly later peak hepatic arterial opacification than that
299 observed using 5ml/s.³⁶ Also, in the present study, both early and delayed post-contrast images
300 were acquired in a cranial to caudal direction whereas the early phase may also be acquired in a
301 caudal to cranial direction.³⁶

302 Variations in the degree of vascular enhancement were evident in the present study. Although
303 acquisition of early phase images always occurred less than 30s after the start of intravenous
304 injection of contrast, there was a wide range of values for aortic attenuation in early phase
305 images. This range will include animals imaged near the peak of aortic enhancement and those

306 imaged after the peak, when portal vein attenuation is rising. Early phase images to examine the
307 liver are acquired after peak arterial enhancement because contrast accumulation representing the
308 hepatic arterial supply to parenchymal lesions will necessarily continue after peak arterial
309 attenuation.^{8,11,14} This is a different technique to that employed for studies focused on vascular
310 anatomy, such as investigation of portosystemic shunting^{37,38}, when it is important to time the CT
311 acquisition to coincide with peak vascular attenuation. The finding that portal attenuation was
312 increased in early phase images in the present study is to be expected because portal attenuation
313 normally exceeds hepatic arterial attenuation within 30s in anaesthetized dogs.³⁶ Conversely, the
314 finding of persistently high aortic attenuation in some delayed phase images suggests that other
315 factors, such as slow circulation time and/or reduced renal excretion of contrast, possibly
316 associated with anesthesia, may have affected some dogs. The rate of change of vascular and
317 parenchymal contrast enhancement is most rapid soon after contrast injection and slows
318 subsequently¹¹, hence variations in timing of delayed phase images may have less effect on
319 contrast accumulation in tissues than variations during the early phase. In the present study,
320 repeating the analyses after removing data for dogs with delayed phase images >240s after
321 administration of contrast did not alter the significance of results of statistical tests. Nevertheless,
322 patient factors and variations in timing of CT image acquisition will have affected the degree of
323 enhancement of masses in post-contrast images, and may have contributed to the overlap
324 observed between CT features of the hepatic and splenic masses in the present study.

325 Automatic triggering of the CT acquisition, based on attenuation within a region of interest (e.g.
326 the abdominal aorta) exceeding a threshold, is a software feature of CT scanners that can be used
327 to increase precision in the timing of post-contrast images. Automatic triggering of the CT
328 acquisition was not utilized for the present study. A test bolus technique has been employed for

329 dual-phase CT studies of dogs with suspected portosystemic shunting^{37,38} and insulinoma.³⁹
330 This technique enables optimal timing of the vascular phases of a multi-phase CT acquisition,
331 but it requires an additional scan and image data analysis³⁶, so is more time-consuming, which is
332 disadvantageous for routine abdominal examinations. Recently, dual-phase CT of conscious
333 sedated and unsedated dogs was described using fixed time delays equal to injection duration for
334 HAP images and 40s for PVP images.⁴⁰ Quality of images obtained was judged to be good or
335 excellent in 72% dogs. Poor quality scans were primarily the result of motion blur or other
336 artifacts; in only one instance was the HAP missed.⁴⁰ Use of fixed time delays is a pragmatic
337 approach for routine abdominal CT studies, although test bolus or automatic triggering methods
338 enable CT scans to be acquired with a more accurately defined vascular phases.

339 On the basis of these results, it may be concluded that the dual-phase protocol we employed for
340 CT of dogs with hepatic or splenic masses provides limited specific diagnostic information.
341 Variability in contrast delivery to masses and relatively low numbers of subjects limit the
342 statistical power of the study. It is possible that significant associations between CT signs and
343 diagnosis would be detected if larger numbers of subjects were studied under more consistent
344 conditions, hence further studies are warranted.

345 Table 1. Size, attenuation, and post-contrast enhancement characteristics of malignant and non-
 346 malignant hepatic masses

	Malignant (n=14)	Non-malignant (n=10)	P-value
Maximum transverse dimension (mm)	61 (15 - 695)	60 (32 - 345)	0.95
Mean hepatic parenchymal attenuation (HU)	60 (53 - 75)	71 (49 - 79)	0.13
Pre-contrast attenuation (HU)	46 (32 - 68)	60 (26 - 69)	0.32
Relative attenuation (HU)	-13 (-28 - -3)	-16 (-35 - -2)	0.75
Early post-contrast attenuation (HU)	83 (42 - 152)	95 (22 - 125)	0.77
Early enhancement (HU)	31 (2 - 113)	42 (-4 - 65)	0.77
Delayed post-contrast attenuation (HU)	77 (42 - 120)	99 (25 - 121)	0.62
Delayed enhancement (HU)	27 (1 - 81)	34 (-1 - 53)	0.98
Contrast accumulation (HU)	-3 (-32 - 20)	-5 (-23 - 52)	0.64

347

348 Values are median (range); HU, Hounsfield units

349 Table 2. Descriptive features of malignant and non-malignant hepatic masses

	Malignant (n=14)	Non-Malignant (n=10)	LR	95% CI
Distinct margins	7 (64%)	4 (36%)	2.0	0.5-3.1
Indistinct margins	7 (54%)	6 (46%)	0.8	0.4-1.7
Homogeneous	8 (67%)	4 (33%)	1.4	0.6-3.5
Heterogeneous	6 (50%)	6 (50%)	0.7	0.3-1.6
Capsular distortion	12 (60%)	8 (40%)	1.1	0.7-1.6
Generalized enhancement	12 (60%)	8 (40%)	1.1	0.7-1.6
Peripheral enhancement	2 (50%)	2 (50%)	0.7	0.1-4.3
Peritoneal fluid	4 (57%)	3 (43%)	0.9	0.3-3.2
Mineralization within mass	3 (75%)	1 (25%)	2.1	0.3-17.7
Abnormal local lymph nodes	11 (65%)	6 (35%)	1.3	0.7-2.3

350

351 LR, Likelihood ratio associated with malignancy

352

353 Table 3. Size, attenuation, and post-contrast enhancement characteristics of malignant and
 354 non-malignant splenic masses

	Malignant (n=18)	Non-malignant (n=13)	P-value
Maximum transverse dimension (mm)	69 (12 - 204)	73 (19 - 173)	0.28
Mean splenic parenchymal attenuation (HU)	53 (19 - 76)	61 (35 - 67)	0.18
Pre-contrast attenuation (HU)	43 (-3 - 55)	49 (12 - 73)	0.12
Relative attenuation (HU)	-6 (-30 - 35)	-2 (-52 - 20)	0.47
Early post-contrast attenuation (HU)	63 (0 - 106)	73 (14 - 119)	0.36
Early enhancement (HU)	20 (0 - 69)	20 (-3 - 64)	0.67
Delayed post-contrast attenuation (HU)	73 (0 - 120)	81 (16 - 112)	0.98
Delayed enhancement (HU)	28 (0 - 82)	30 (4 - 57)	0.50
Contrast accumulation (HU)	10 (-11 - 56)	3 (-7 - 27)	0.56

355

356 Values are median (range); HU, Hounsfield units

357

358 Table 4. Descriptive features of malignant and non-malignant splenic masses

	Malignant (n=18)	Non-Malignant (n=13)	LR	95% CI
Distinct margins	6 (43%)	8 (57)	0.5	0.2-1.2
Indistinct margins	12 (71%)	5 (29%)	1.7	0.8-3.7
Homogeneous	5 (56%)	4 (44%)	0.9	0.3-2.7
Heterogeneous	13 (59%)	9 (41%)	1.0	0.6-1.7
Capsular distortion	17 (59%)	12 (41%)	1.0	0.8-1.2
Generalized enhancement	16 (57%)	12 (43%)	0.9	0.8-1.2
Peripheral enhancement	2 (67%)	1 (33%)	1.4	0.1-14.3
Peritoneal fluid	12 (60%)	8 (40%)	1.1	0.6-1.9
Mineralization within mass	1 (100%)	0 (0%)	2.2	0.1-50.3
Abnormal local lymph nodes	6 (55%)	5 (45%)	0.9	0.3-2.2

359

360 LR, Likelihood ratio associated with malignancy

361

362 Table 5. Pre- and post-contrast features of hepatocellular carcinoma, adenoma, and nodular
 363 hyperplasia

	Hepatocellular carcinoma (n=9)	Adenoma (n=6)	Nodular hyperplasia (n=3)
Pre Contrast			
Hypoattenuation	5 (56%)	5 (83%)	2 (67%)
Isoattenuation	4 (44%)	1 (17%)	1 (33%)
Homogenous	3 (33%)	2 (33%)	2 (67%)
Heterogeneous	6 (66%)	4 (66%)	1 (33%)
Early Phase			
Slight enhancement	2 (22%)	1 (17%)	2 (67%)
Marked enhancement	7 (78%)	5 (83%)	1 (33%)
Generalized	7 (78%)	6 (100%)	2 (67%)
Peripheral	2 (22%)	0	1 (33%)
Late Phase			
Slight enhancement	2 (22%)	0	2 (67%)
Marked enhancement	7 (78%)	6 (100%)	1 (33%)
Contrast accumulation	2 (22%)	0	1 (33%)
Lack of contrast accumulation	7 (78%)	6 (100%)	2 (67%)

364

365 Table 6. Pre- and post-contrast features of splenic hemangiosarcoma, hematoma, and nodular
 366 hyperplasia

	Hemangiosarcoma (n=13)	Hematoma (n=8)	Nodular hyperplasia (n=5)
Pre Contrast			
Hypoattenuation	4 (31%)	2 (25%)	3 (60%)
Isoattenuation	8 (62%)	5 (63%)	2 (40%)
Hyperattenuation	1 (7%)	1 (12%)	0
Homogenous	4 (31%)	3 (38%)	1 (20%)
Heterogeneous	9 (69%)	5 (62%)	4 (80%)
Early Phase			
Slight enhancement	2 (15%)	5 (62%)	0
Marked enhancement	11 (85%)	3 (38%)	5 (100%)
Generalized	12 (92%)	7 (88%)	5 (100%)
Peripheral	1 (7%)	1 (12%)	0
Late Phase			
Slight enhancement	1 (7%)	3 (38%)	0
Marked enhancement	12 (92%)	5 (62%)	5 (100%)
Contrast accumulation	10 (77%)	8 (100%)	2 (40%)
Lack of contrast accumulation	3 (23%)	0	3 (60%)

367

368 **References**

- 369 1. Patnaik AK, Hurvitz AI, Lieberman PH, Johnson GF. Canine hepatocellular carcinoma.
370 Vet Pathol 1981; 18: 427-438.
- 371 2. Balkman C. Hepatobiliary neoplasia in dogs and cats. Vet Clin North Am: Small Anim
372 Pract 2009; 39: 617–625.
- 373 3. Prymak C, McKee LJ, Goldschmidt MH, Glickman LT. Epidemiologic, clinical,
374 pathologic, and prognostic characteristics of splenic hemangiosarcoma and splenic
375 hematoma in dogs: 217 cases (1985). J Am Vet Med Assoc 1988; 193:706-712.
- 376 4. Spangler WL, Culbertson MR. Prevalence, type, and importance of splenic diseases in
377 dogs: 1,480 cases (1985-1989). J Am Vet Med Assoc 1992; 200: 829-834.
- 378 5. Bergman JR. Nodular hyperplasia in the liver of the dog: an association with changes in
379 the Ito cell population. Vet Pathol 1985; 22: 427-438.
- 380 6. Stowater JL, Lamb CR, Schelling SH. Ultrasonographic features of canine hepatic
381 nodular hyperplasia. Vet Radiol 1990;31:268-272.
- 382 7. Johnson KA, Powers BE, Withrow SJ, Sheetz MJ, Curtis CR, Wrigley RH.
383 Splenomegaly in dogs. Predictors of neoplasia and survival after splenectomy. J Vet
384 Internal Med 1989; 3:160-166.
- 385 8. Sahani DV, Singh AH. Dual-phase liver MDCT. In: Kalra MK, Saini S, Rubin GD
386 (Eds). MDCT From Protocol to Practice. Milan: Springer, 2008, pp83-92.
- 387 9. Ji H, McTavish JD, Morteale KJ, Wiesner W, Ros PR. Hepatic imaging with
388 multidetector CT. Radiographics 2001;21:S71-S80.
- 389 10. Miles, KA. Tumour angiogenesis and its relation to contrast enhancement on computed
390 tomography: a review. Eur J Radiol 1999; 30:198-205.

- 391 11. Bae KT. Principles of contrast medium delivery and scan timing in MDCT. In: Kalra
392 MK, Saini S, Rubin GD (Eds). MDCT From Protocol to Practice. Milan: Springer,
393 2008, pp10-24.
- 394 12. Hwang GJ, Kim MJ, Yoo HS, Lee JT. Nodular hepatocellular carcinomas: Detection
395 with arterial-, portal-, and delayed-phase images at spiral CT. Radiology 1997;
396 202:383-388.
- 397 13. Murakami T, Kim T, Takamura M, et al. Hypervascular hepatocellular carcinoma:
398 detection with double arterial phase multidetector helical CT. Radiology 2001;
399 218:763-767.
- 400 14. Oliver JH, Baron RL, Federle MP, Jones BC, Sheng R. Hypervascular liver metastases:
401 do unenhanced and hepatic arterial phase CT images affect tumor detection? Radiology
402 1997; 205:709-715.
- 403 15. Ichikawa T, Kitamura T, Nakajima H, Sou H, Tsukamoto T, Ikenaga S, et al.
404 Hypervascular hepatocellular carcinoma: can double arterial phase imaging with
405 multidetector CT improve tumor depiction in the cirrhotic liver? Am J Roentgenol
406 2002;179:751-758.
- 407 16. Baron RL, Oliver JH, Dodd GD, Nalesnik M, Holbert BL, Carr B. Hepatocellular
408 carcinoma: evaluation with biphasic, contrast-enhanced, helical CT. Radiology 1996;
409 199:505-511.
- 410 17. Bae KT. Intravenous contrast medium administration and scan timing at CT:
411 considerations and approaches. Radiology 2010;256:32-61.
- 412 18. Ichikawa T, Erturkb SM, Arakia T. Multiphasic contrast-enhanced multidetector-row
413 CT of liver: Contrast-enhancement theory and practical scan protocol with a
414 combination of fixed injection duration and patients' body-weight-tailored dose of

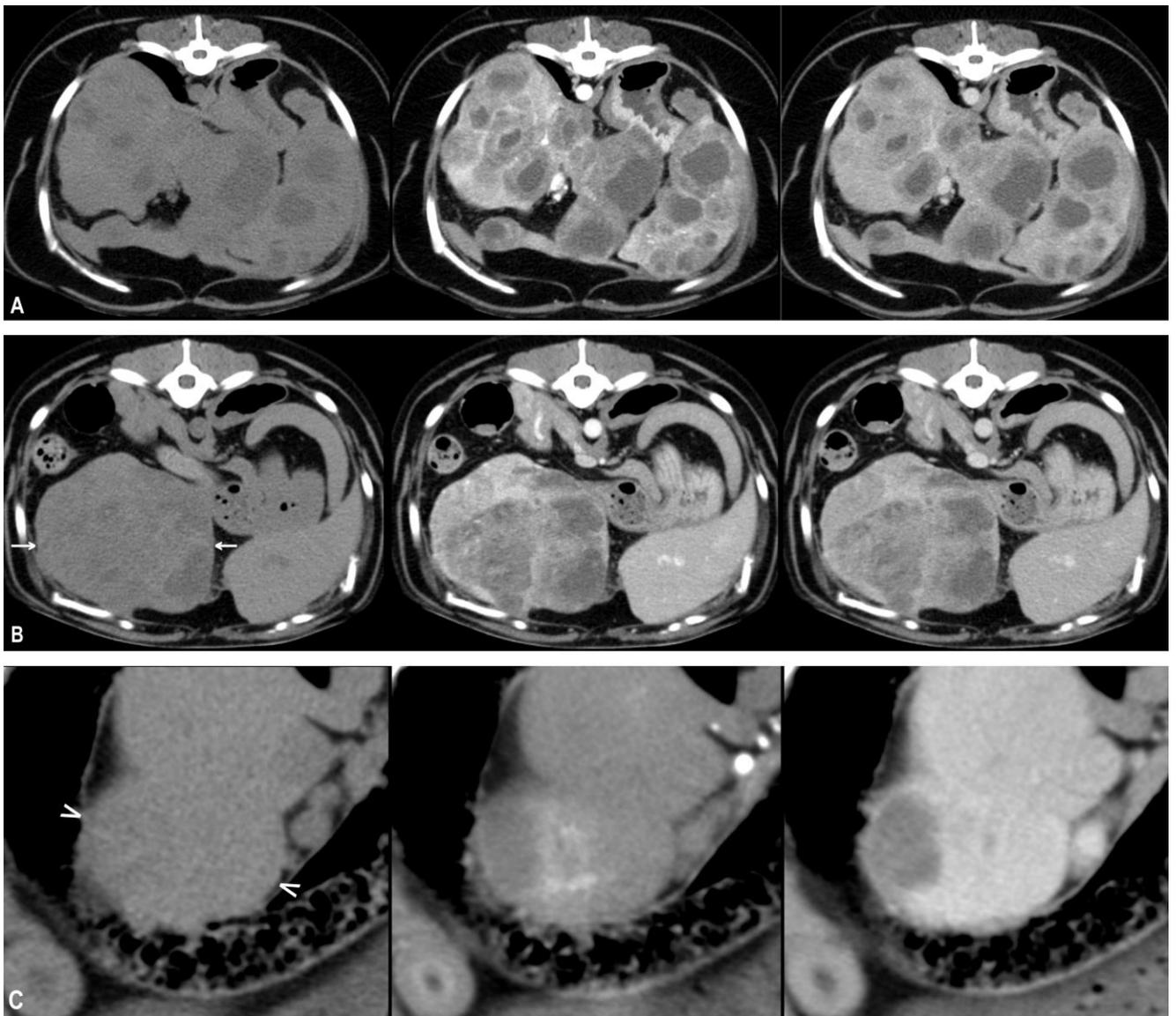
- 415 contrast material. *Eur J Radiol* 2006;58: 165–176.
- 416 19. Chan MG, Cassidy FH, Andre MP, Chu P, Aganovic L. Delayed imaging in routine CT
417 examinations of the abdomen and pelvis: is it worth the additional cost of radiation and
418 time? *Am J Roentgenol* 2014;202:329-335.
- 419 20. Vasanawala SS, Desser T. Value of delayed imaging in MDCT of the abdomen and
420 pelvis. *Am J Roentgenol* 2006;187:154-163
- 421 21. Fukushima K, Hideyuki K, Ohno K, Takahashi M, Nakashima K, Fujino Y, et al. CT
422 Characteristics of primary hepatic mass lesions in dogs. *Vet Radiol Ultrasound* 2012;
423 53: 252–257.
- 424 22. Kutara K, Seki M, Ishikawa C, Sakai M, Kagawa Y, Iida G, et al. Triple-phase helical
425 computed tomography in dogs with hepatic masses. *Vet Radiol Ultrasound* 2014; 55:7–
426 15.
- 427 23. Fife WD, Samii VF, Drost T, Mattoon JS, Hoshaw-Woodard S. Comparison between
428 malignant and nonmalignant splenic masses in dogs using contrast enhanced computed
429 tomography. *Vet Radiol Ultrasound* 2004; 45: 289–297.
- 430 24. Beukers M, Vilaplana Grosso F, Voorhout G. Computed tomographic characteristics of
431 presumed normal canine abdominal lymph nodes. *Vet Radiol Ultrasound* 2013; 54:
432 610–617.
- 433 25. Cullen JM, Brown DL. Hepatobiliary system and exocrine pancreas. In: Zachery JF,
434 McGavin MD (Eds). *Pathologic Basis of Veterinary Disease*, 5th edition. St. Louis:
435 Elsevier, 2012, pp442-445.
- 436 26. Stalker MJ, Hayes MA. Liver and biliary system. In: Maxie MG (Ed). *Jubb, Kennedy,*
437 *and Palmer’s Pathology of Domestic Animals*, 5th edition, vol.2. St. Louis: Elsevier,
438 2007, pp382-388.

- 439 27. Gregori T, Mantis P, Benigni L, Priestnall S, Lamb CR. Comparison of computed
440 tomographic and pathologic findings in 17 dogs with primary adrenal neoplasia. *Vet*
441 *Radiol Ultrasound* 2015;56:153-159.
- 442 28. Fukuda S, Kobayashi T, Robertson ID, Oshima F, Fukazawa E, Nakano Y, et al.
443 Computed tomographic features of canine nonparenchymal haemangiosarcoma. *Vet*
444 *Radiol Ultrasound* 2014; 55: 374–379.
- 445 29. Day MJ, Lucke VM, Pearson H. A review of pathological diagnoses made from 87
446 canine splenic biopsies. *J Small Anim Pract* 1995; 36: 426-433.
- 447 30. Maxie MG, Robinson WF. Cardiovascular system. In: Maxie MG (Ed). *Jubb, Kennedy,*
448 *and Palmer’s Pathology of Domestic Animals*, 5th edition, vol.3. St. Louis: Elsevier,
449 2007, pp102-105.
- 450 31. Mallinckrodt MJ, Gottfried SD. Mass-to-splenic volume ratio and splenic weight as a
451 percentage of body weight in dogs with malignant and benign splenic masses: 65 cases
452 (2007-2008). *J Am Vet Med Assoc* 2011;239:1325-1327.
- 453 32. Taeymans O, Penninck DG. Contrast enhanced sonographic assessment of feeding
454 vessels as a discriminator between malignant vs. benign focal splenic lesions. *Vet*
455 *Radiol Ultrasound* 2011; 52:457–461.
- 456 33. Rossi F, Leone VF, Vignoli M, Laddaga E, Terragni R. Use of contrast-enhanced
457 ultrasound for characterization of focal splenic lesions. *Vet Radiol Ultrasound*
458 2008;49:154-164.
- 459 34. Ivancic M, Long F, Seiler GS. Contrast harmonic ultrasonography of splenic masses
460 and associated liver nodules in dogs. *J Am Vet Med Assoc* 2009;234: 88-94.
- 461 35. Nakamura K, Sasaki N, Murakami M, Kumara WRB, Ohta H, Yamasaki M, et al.
462 Contrast-enhanced ultrasonography for characterization of focal splenic lesions in dogs.

- 463 J Vet Internal Med 2010;24:1290-1297.
- 464 36. Zwingenberger AL, Schwartz T. Dual-phase CT angiography of the normal canine
465 portal and hepatic vasculature. Vet Radiol Ultrasound 2004; 45: 117-124.
- 466 37. Zwingenberger AL, Schwarz T, Saunders HM. Helical computed tomographic
467 angiography of canine portosystemic shunts. Vet Radiol Ultrasound 2005;46: 27-32.
- 468 38. Park K, Yeon S, Lee H. Hepatic arteriovenous fistula in four dogs: usefulness of dual-
469 phase computed tomographic angiography. J Biomed Res 2012;13:195-199.
- 470 39. Mai W, Cáceres AV. Dual-phase computed tomographic angiography in three dogs
471 with pancreatic insulinoma. Vet Radiol Ultrasound 2008; 49: 141–148.
- 472 40. Shanaman MM, Hartman SK, O'Brien RT. Feasibility for using dual-phase contrast-
473 enhanced multi-detector helical computed tomography to evaluate awake and sedated
474 dogs with acute abdominal signs. Vet Radiol Ultrasound 2012;53: 605–612.
- 475

476 **Legends**

477 Figure 1. Examples of dual-phase CT images of hepatic masses in dogs. A) Hepatocellular carcinoma; B)
478 adenoma (between arrows); C) nodular hyperplasia (between arrowheads). In each instance images are pre-
479 contrast (left), early phase (middle) and delayed phase (right). Each of these lesions has slightly lower
480 attenuation and is heterogeneous compared to surrounding hepatic parenchyma in pre-contrast images, and show
481 marked, generalized enhancement in the early phase images that persists in delayed phase images. Multiple
482 masses with similar features are present throughout the liver in the dog with hepatocellular carcinoma. Window
483 350HU, level 50HU.



484

485

