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1	Ultrasonographically visible hepatic location in clinically normal horses
2	IC Johns* and A Miles
3	Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire,
4	AL97TA, UK
5	*Email: <u>ijohns@rvc.ac.uk</u> . Address as above
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7	The results of this study were presented at the Annual Forum of the American College of
8	Veterinary Internal Medicine, 2014, Nashville TN
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### 20 Abstract:

21 **Objective**: Hepatic ultrasound is widely used for evaluating horses with suspected liver dysfunction. Although a change in size is considered suggestive of pathology, no clear 22 23 guidelines exist to define the hepatic ultrasonographically visible locations (HUVL) in horses. The aim of the study was to describe the HUVL in normal horses, and determine 24 whether this is altered by signalment, height, weight and body condition score (BCS). 25 Design: Prospective observational study. Procedure: Bilateral ultrasonographic evaluation 26 was performed in 58 clinically normal horses with no history of hepatic disease. The most 27 cranial/caudal intercostal spaces (ICS), total number of ICS in which liver was visualized and 28 29 the ventral extent of the liver was recorded. **Results:** Liver was visualized on the right in 56/58 horses (97%), the left in 41/58 (71%) and on both sides in 39/58 (67%). The most 30 cranial ICS was 5 (right) or 4 (left) and the most caudal 16 (right) and 11 (left). Liver was 31 32 visualized in 0-11 ICS (right) and 0-5 ICS (left). Liver was not visualized ventral to the costochondral junction. There was no significant effect of sex, breed, height, weight or BCS 33 34 on HUVL. Liver was visible in significantly fewer ICS on the right in horses aged 24 years and older compared to younger horses (median 3.5 vs 7 ICS; p=0.016). Conclusion: These 35 findings suggest that the liver should be consistently visualized on the right side, but absence 36 37 of ultrasonographically visible liver on the left is unlikely to be clinically relevant. Liver dimensions may be decreased in older horses. 38

# 39 Key words: liver; imaging; size; atrophy

#### 40 Abbreviations:

41 HUVL: hepatic ultrasonographically visible locations

42 ICS: intercostal space

### 43 Introduction

Ultrasonographic examination of the liver is widely utilised in the investigation of horses 44 with suspected hepatic disorders. Other imaging modalities such as computed tomography 45 and magnetic resonance imaging, which are routinely employed in people and small animals 46 such as cats and dogs cannot be performed on adult horses because of their size. In addition, 47 the liver cannot be palpated either per rectum or via transabdominal palpation. As such, 48 hepatic ultrasound has become a key tool in the investigation of hepatic disorders in horses.<sup>1-4</sup> 49 Ultrasound is used to evaluate hepatic echogenicity, for the presence of masses and to assess 50 hepatic size.<sup>1-5</sup> Although the sensitivity of ultrasound for detecting hepatic disease is low 51 (26%), the specificity and positive predictive value are relatively high (86% and 85% 52 respectively) suggesting that when hepatic abnormalities are present ultrasonographically, 53 they correlate to organ dysfunction.<sup>5</sup> An increase in hepatic size as determined by 54 55 ultrasonographic examination has been reported in conjunction with a number of different disorders including neoplasia, cholangiohepatitis and cholelithiasis, hepatic lipidosis, non-56 neoplastic masses, liver lobe torsion and Tyzzer's disease.<sup>3,4,6,7</sup> A smaller than normal liver 57 has been reported secondary to hepatic fibrosis, cholelithiasis and Theiller's disease.<sup>3,4</sup> Right 58 liver lobe atrophy has been described as a post mortem finding in a group of horses aged 5-30 59 years, and was hypothesized to be secondary to chronic colonic compression of the hepatic 60 parenchyma.<sup>8</sup> Although hepatic ultrasound findings were not reported in this study, a 61 decrease in the size of the right hepatic lobe identified via ultrasound examination has been 62 described as a normal finding in older horses.<sup>3,4</sup> Data to support this observation has not, to 63 the authors' knowledge, been presented, nor the age at which this finding might occur. 64

Despite hepatic size being a key factor in the ultrasonographic evaluation of the liver,
reference ranges for ultrasonographically visible hepatic dimensions have not been

determined in horses, and the majority of assessments are made based on a subjective
evaluation of hepatic size.<sup>3,4</sup>

The lack of defined hepatic ultrasonographic dimensions means that assessment of hepatic size remains subjective. Documenting the location of the left and right liver lobes could potentially provide more objective information for clinicians attempting to assess liver size in the horse. The aims of this study were thus to (1) Determine the ultrasonographically visible hepatic location (based on visibility within each ICS) in clinically normal horses; and (2) Determine whether these locations are altered by age, breed, sex, weight, body condition score and height.

# 76 Materials and Methods

The study was approved by the Ethics and Welfare Committee of the Royal Veterinary 77 78 College and informed owner consent was obtained prior to examination. Percutaneous ultrasonographic examination was performed on 58 clinically normal horses with no history 79 of hepatic disease. Horses were recruited from three separate locations including an equine 80 charity, a facility for retired working horses and an equine referral hospital population. 81 Horses with no clinical evidence or history of hepatic disease were included. Horses with a 82 history of hepatic disease, or those being evaluated in the referral hospital for gastrointestinal 83 disease were excluded. The age, breed and sex of each horse were recorded. Prior to 84 examination each horse's weight was recorded, height at the withers measured in centimeters 85 and body condition score (BCS) assigned utilising the Henneke system.<sup>9</sup> The feeding and 86 87 management routines varied according to location and individual horse, and horses were not 88 withheld from food or water prior to examination.

89 Ultrasound examination was performed using either a MyLab<sup>TM</sup> 30 VET Gold (Esaote

90 Group, Genova, Italy) or a Vivid 7 Dimension (GE Healthcare, Hatfield, Herts UK) using a

91 2.5MHz phased-array transducer at a frequency of 3.5 MHz.

The examinations were performed during the summer months, and horses were not clipped or 92 sedated for the procedure. Contact was enhanced by ensuring coats were free of dirt and dust 93 followed by liberal application of surgical spirit. The examination was performed on both 94 sides of the abdomen starting in the paralumbar fossa and progressing cranially in each 95 intercostal space. In each space, the presence or absence of the liver was noted following 96 evaluation in longitudinal section from the dorsal aspect of the abdominal cavity as 97 determined by the characteristic appearance of gastrointestinal viscera and/or the presence of 98 the pleural reflection to a point ventral to the costo-chondral junction. The probe was 99 maintained perpendicular to the horse's skin and was not angled in a cranial or caudal manner 100 101 within each ICS. The presence or absence of the liver in each ICS was determined in real time by an experienced ultrasonographer based on the characteristic ultrasonographic 102 appearance as previously described.<sup>3,4,10</sup> The total number of ICS in which the liver could be 103 identified, the most cranial and the most caudal intercostal space in which the liver could be 104 visualised, and whether the ventral margin of the liver extended ventral to the costochondral 105 106 junction was noted.

For the purposes of the analysis, horses were further categorised based on height (horses
150cm and above; ponies below 150cm); weight (less than 250kg; 251-500kg; greater than
501kg) breed: (pony breeds; Cobs and cob crosses; Thoroughbred/Warmbloods or crosses),
and BCS: underweight (1-4) ideal (5-6) overweight (7-9). As there is no definition for the age
at which horses are considered 'older' and thus have an increased likelihood of right liver
lobe atrophy, three age categories were utilised. These were based on 2 different definitions

for geriatric horses (older than 20 years; older than 15 years) as well as a category that
defined 'older' horses as the oldest 10% of the study population (in this case 24 years and
older).<sup>11-14</sup>

# 116 Statistical analysis

Statistical tests were selected and performed by one of the authors (IJ) using a commercially 117 available software package (SPSS 20 for Windows; SPSS Inc, Chicago, IL) and significance 118 was assumed at P<0.05. As all data apart from body weight were not normally distributed, 119 the median (± interquartile range [IQR]) is displayed for each measurement. The body weight 120 is expressed as mean (± standard deviation [SD]). The total number of ICS, and the most 121 cranial and caudal ICS in which the liver could be visualized on both sides of the abdomen 122 123 were compared between age, sex, height, breed, and BCS categories using the Mann Whitney U or the Kruskal-Wallis test as appropriate. A Spearman's/Pearson's rank order correlation 124 was performed to determine whether there was a correlation between horse age, weight, BCS 125 126 and height, and the total ICS, most cranial and most caudal ICS in which the liver was 127 visualized on both sides of the abdomen. Finally, the proportion of horses in each category (age, weight, breed, sex, BCS and height) in which the liver could be visualized at each ICS 128 was compared using the Chi-squared test for independence. 129

130

# 131 **Results**

Ultrasonographic evaluation of the liver was performed in 58 horses representing a wide
range of ages, heights, weights, breeds and body condition scores (Table 1). There were 34
male horses (33 geldings and one colt) and 24 mares. The total number of horses within each
category is shown in Table 2.

136 The liver could be visualized in at least one ICS on the right in 56/58 (97%) of horses, on the left in 41/58 (71%) and on both sides in 39/58 (67%). The most cranial ICS in which the liver 137 could be visualised was ICS 5 (right) and ICS 4 (left) and the most caudal was ICS 16 (right) 138 139 and ICS 11 (left). The number of ICS in which the liver could be visualized ranged from 0 to 11 ICS (median 6.5; IQR 3) on the right side and from 0 to 5 ICS (median 2; IQR 3) on the 140 left. On the right, liver was most consistently visualized in the 12<sup>th</sup> (53/58 horses), 13<sup>th</sup> 141 (55/58) and 14<sup>th</sup> ICS (54/58; Figure 1). The intercostal spaces on the left where liver could 142 most consistently be identified were ICS 6 (27/58) and ICS 7 (30/58; Figure 2). The liver did 143 144 not extend ventral to the costochondral junctions in any horse. There was no significant effect of sex, breed, height, body weight or BCS on the HUVL. The liver was visible in 145 significantly fewer ICS on the right (median 3.5) in horses aged 24 years and older compared 146 147 to younger horses (median 7 ICS; p=0.016). (Tables 3a and b)

When the proportion of horses in each age, sex, breed, height, weight and BCS categories in which liver could be visualised ultrasonographically at each ICS was compared, there was no statistically significant difference identified. There was also no significant correlation between horse age, weight, BCS and height, and the total ICS, most cranial and most caudal ICS in which the liver was visualized on both sides of the abdomen.

# 153 Discussion

154 To the authors' knowledge, this is the first study that has detailed the anatomical

155 ultrasonographic position of the liver on both sides of the abdomen in clinically normal

horses. A previous report, published over 20 years ago when equine ultrasonography was in

its infancy, described the liver as being visible between the 9<sup>th</sup> and 16<sup>th</sup> ICS on the right, but

did not describe whether it was visible on the left.<sup>15</sup> In the current study, the liver could be

seen as far cranially as ICS 5 on the right hand side, albeit in only a small proportion of

160 horses, and could be seen in 71% of horses from the left hand side. These findings are more consistent with more recent suggestions that the liver is usually visible on the right between 161 8-15 ICS and on the left between 7-10 ICS.<sup>10</sup> In no horses was the liver visible ventral to the 162 costochondral junctions, supporting the suggestion that this finding would be consistent with 163 hepatomegaly.<sup>3,4,</sup> A recent study investigated the frequency with which the liver could be 164 visualized ultrasonographically at sites where a blind percutaneous liver biopsy would be 165 performed.<sup>2</sup> As a result, only the right side of the abdomen was evaluated and evaluation of 166 the entire ICS in a dorso-ventral manner was not performed as the authors were specifically 167 168 interested in biopsy site location. As such, the findings of this study cannot be directly compared to the current study, although in both studies, the liver was most frequently 169 visualized in the 13<sup>th</sup> and 14<sup>th</sup> ICS on the right hand side. 170

In people, hepatic size can be affected by factors such as body mass index (BMI), age and 171 172 gender, with reference ranges for normal liver size differing between patients depending on these characteristics.<sup>16,17,18</sup> An increased liver size is seen with higher BMI and in men. A 173 174 similar finding was not however identified in the current study, although the method for the assessment of liver size differed from human studies. In people, ultrasonographic 175 measurement of liver size as measured by depth of parenchyma at the midclavicular line has 176 been shown to be a good estimate of hepatic size, although computed tomographic (CT) 177 scanning and the calculation of liver volume is considered a more precise measure.<sup>16,19</sup> In the 178 authors' experience, ultrasonographic identification of the liver in obese horses can be 179 difficult, although the findings of the study do not support this clinical impression as there 180 was no difference in the number of ICS in which liver could be identified on either side of the 181 abdomen, regardless of body condition score. However, the number of horses with BCS of 8 182

or 9 was low (1/58 with BCS 9; 0/58 with BCS of 8) and examination of a larger number of
obese horses may provide additional information to support or refute this clinical impression.

The effect of age on the ultrasonographically visible hepatic dimensions was of particular 185 interest. Right liver lobe atrophy is frequently described as a common and normal finding in 186 'older' horses, with some authors stating that 'little or none' of the right liver lobe is imaged 187 on the right in normal older horses.<sup>3</sup> The age at which horses are considered 'older' and thus 188 when this would be considered a normal finding has not to the authors' knowledge been 189 190 determined, nor has evidence to support this clinical impression been provided. In the only post mortem study reporting right liver lobe atrophy, the ages of the horses ranged from 5 to 191 30 years the mean age was 12.6 years.<sup>8</sup> The majority of these horses (15/17) had a history of 192 abdominal pain, and the author hypothesized that a diet high in concentrates may have 193 contributed to atony of the right dorsal colon with resulting distention that then compressed 194 195 the right liver lobe. An expected decrease in liver weight determined at autopsy has been described with increasing age in people, although there is some disagreement as to whether 196 this change in size can be detected ultrasonographically.<sup>16,20,21</sup> 197

In the current study, the two horses in which liver could not be identified on the right hand 198 199 side were aged 42 and 28 years, which may support the age related decrease in size of the right liver lobe described by various authors.<sup>3,4</sup> As described, the age categories chosen for 200 comparative purposes were based on studies in geriatric horses and using these categories no 201 statistical differences in the liver dimensions based on age could be identified.<sup>11-14</sup> It was only 202 when the oldest 10% of horses in the study (older than 24 years) were compared to younger 203 204 horses that a difference in the number of ICS where liver could be visualized on the right was identified. As there were only 6 horses in this 'older' group, it is unknown whether this 205 difference would remain in a larger group of horses. It is unlikely that determination of the 206

number of ICS in which the liver can be visualized is a sensitive enough measure to identifymore subtle hepatic atrophy.

In people, ultrasonographic measurement of liver size based on measurement at the 209 midclavicular line has been shown to be a good estimate of hepatic size.<sup>16</sup> The measurement 210 is obtained with patients in the supine position with right hand placed behind the head to 211 optimise hepatic visualisation. Whether an adaptation of this method could be used to assess 212 hepatic size in horses is unknown, although hepatic depth measurements have been reported 213 in cows and goats with the portal vein and caudal vena cava used as landmarks.<sup>22,23</sup> An 214 increase in pulmonary volume has been reported in horses with chronic lung diseases such as 215 Recurrent Airway Obstruction.<sup>24</sup> Whether this could explain an inability or impaired ability to 216 visualise the liver ultrasonographically is unknown. Neither of the horses in this report had 217 any known respiratory disease, although whether the ability to visualise the liver could be 218 219 improved by implementation of a rebreathing examination is unknown, as it was not performed in either case. 220

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Whilst the horses in this study did not display any clinical signs consistent with hepatic 222 disease, because biochemical analysis of venous blood was not performed the presence of 223 subclinical hepatic disease cannot be ruled out. Although the horses were relatively evenly 224 distributed within most categories, lighter horses, those with a low (1-4) or a high (8-9) BCS 225 and older horses (>24 years) were relatively underrepresented within their respective 226 227 categories. Whether a more even distribution of horses would have affected the results is unknown. Finally, although the purpose of the study was to determine the hepatic 228 229 ultrasonographically visible location, this is a relatively crude measure of hepatic size and does not take into consideration the potential for cranial or caudal displacement secondary to 230

the presence of pathology in adjacent visceral structures (eg. colonic dilation).

232 Ultrasonographic examination including various measurements of the depth of hepatic

233 parenchyma, followed by post mortem examination to determine actual hepatic dimensions

would be required to provide further information regarding the normal hepatic

235 ultrasonographic size in horses, and thus whether ultrasound can be used to more accurately

determine a change in hepatic size in horses with hepatic disease.

### 237 Conclusion

238 The findings of this study provide information to guide clinicians as to the hepatic location

visible on ultrasonographic examination in clinically normal horses. The liver can be

expected to be seen on the right hand side most commonly in ICS 12, 13 and 14, and on the

left in ICS 6 and 7. The number of ICS in which the liver can be visualised may be lower in

older horses, although other characteristics do not appear to affect the HUVL.

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# 315 Tables

Characteristic	Median	Minimum to
	(IQR)	Maximum
Age (years)	14.5	1-42
	(13)	
Height (centimetres)	152.4	83.8-180.3
	(34.3)	
Weight (kg)	461.7kg	115-918
*mean (Std Dev)	(187.8)	
Body condition score (1-9)	5	2-9
	(2)	

316 Table 1: Descriptive Data of 58 Clinically Normal Horses Enrolled in Study

317

Table 2: Total Number of Horses in each Category. Cobs/X: Cobs or Cob crosses; TB/WB/X:

320 Thoroughbreds, Warmbloods or crosses; BCS: out of 9

Age (years)	Breed	Sex	Height (cm)	Weight (kg)	BCS
Category; N					
≤20; 40	Ponies; 20	Male; 34	≥150cm; 30	<250; 11	1-4; 11
20; 18	Cobs/X; 16	Female; 24	<150cm; 28	251-500; 21	5-6; 35
	TB/WB/X; 22			>501; 26	7-9; 12
≤15; 30					
>15; 28					
<24; 52					
≥24; 6					

324 Table 3a: Comparison of median number of ICS, the most cranial and the most caudal ICS on the right in which

325 liver could be visualised ultrasonographically based on age, sex, breed, height, weight and BCS categories.

P<0.05. TB: Thoroughbred. WB: Warmblood. X: cross breed. ICS: Intercostal space. BCS: Body condition</li>
 score (1-9)

Total ICS right				P value
Age category	≤20y	>20y		0.622
Median No. ICS	6	7		
Age	≤15y	>15y		0.399
Median No. ICS	7	6		
Age	<24y	≥24y		$0.016^{*}$
Median No. ICS	7	3.5		
Sex	Male	Female		0.442
Median No. ICS	7	6		
Breed category	Pony	Cob/X	TB/WB/X	0.556
Median No. ICS	6	7	7	
Height	Horse	Pony		0.620
Median No. ICS	7	6		
Weight category	≤250kg	251-500kg	>500kg	0.780
Median No. ICS	6	6	7	
BCS category	1-4	5-6	7-9	0.556
Median No. ICS	6	6	7	
Most cranial ICS				P value
Age category	≤20y	>20y		0.193
Median No. ICS	9	8		
Age	≤15y	>15y		0.914
Median No. ICS	8.5	9		
Age	<24y	$\geq 24y$		0.911
Median No. ICS	9	9		
Sex	Male	Female		0.266
Median No. ICS	8	9		
Breed category	Pony	Cob/X	TB/WB/X	0.815
Median No. ICS	9	9	8	
Height	Horse	Pony		0.670
Median No. ICS	8.5	9		
Weight category	≤250kg	251-500kg	>500kg	0.517
Median No. ICS	10	8	8.5	
BCS category	1-4	5-6	7-9	0.590
Median No. ICS	8	9	8	
M				D 1
Most caudal ICS	<20.	>20v		P value
Age category Modion No. ICS	$\geq 20y$	≥20y 15		01/3
A co	14 ~15	13		0.757
Age Madian Na ICC	≤13y	>15y		0.757
Median No. ICS	14	14		0 175
Age	<24y	≥24y		0.1/5
Median No. ICS	14	14		0.447
Sex	Male	Female		0.447
Median No. ICS	2	1.5		0.0==
Breed category	Pony	Cob/X	TB/WB/X	0.877
Median No. ICS	14	14.5	14	
Height	Horse	Pony		0.904
Median No. ICS	14	14		
Weight category	$\leq 250 \text{kg}$	251-500kg	>500kg	0.657
Median No. ICS	15	14	14	

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**BCS category** Median No. ICS 5-6

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0.692

328 Table 3b: Comparison of median number of ICS, the most cranial and the most caudal ICS on the left in which

329 liver could be visualised ultrasonographically based on age, sex, breed, height, weight and BCS categories.

P<0.05 Thoroughbred. WB: Warmblood. X: cross breed. ICS: Intercostal space. BCS: Body condition score (1-</li>
 9)

Total ICS left				P value
Age category	≤20y	>20y		0.380
Median No. ICS	1.5	2		
Age	≤15y	>15y		0.591
Median No. ICS	1.5	2		
Age	<24y	≥24y		0.249
Median No. ICS	1.5	2.5		
Sex	Male	Female		0.447
Median No. ICS	2	1.5		
Breed category	Pony	Cob/X	TB/WB/X	0.109
Median No. ICS	2	1	2	
Height	Horse	Pony		0.892
Median No. ICS	2	1.5		
Weight category	≤250kg	251-500kg	>500kg	0.678
Median No. ICS	1	2	2	
BCS category	1-4	5-6	7-9	0.167
Median No. ICS	3	1	1	
Most cranial ICS				P value
Age category	≤20y	>20y		0.455
Median No. ICS	6	6		
Age	≤15y	>15y		0.100
Median No. ICS	6	6		
Age	<24y	≥24y		0.676
Median No. ICS	6	6		
Sex	Male	Female		0.429
Median No. ICS	6	6		
Breed category	Pony	Cob/X	TB/WB/X	0.430
Median No. ICS	5.5	6	6	
Height	Horse	Pony		0.262
Median No. ICS	6	6		
Weight category	≤250kg	251-500kg	>500kg	0.755
Median No. ICS	6	6	6	
BCS category	1-4	5-6	7-9	0.190
Median No. ICS	5	6	6	
Most caudal ICS				P value
Age category	≤20y	>20y		0.460
Median No. ICS	7	8		
Age	≤15y	>15y		0.790
Median No. ICS	7	7		
Age	<24y	≥24y		0.458
Median No. ICS	7	7		
Sex	Male	Female		0.529
Median No. ICS	7.5	7		0.05
Breed category	Pony	Cob/X	TB/WB/X	0.094
Median No. ICS	7	7	8	
Height	Horse	Pony		0.070
Median No. ICS	8	7		
Weight category	≤250kg	251-500kg	>500kg	0.313
Median No. ICS	7	7	8	
BCS category	1-4	5-6	7-9	0.858
Median No. ICS	7	7.5	8	

Figure 1: Percentage of 58 horses in which liver could be visualized via ultrasonography at 





Figure 2: Percentage of 58 horses in which liver could be visualized via ultrasonography at each intercostal space on the left.

