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## Detection of Hepatic Problem in Dogs and Cats by Biochemical Assay and Imaging Technique (X-Ray and Ultrasonography)

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#### 6 Abstract

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7 Keeping pet (dogs and cats) has become a cultural phenomenon throughout the world

<sup>8</sup> including Bangladesh. Hepatic problem is an important health issue that may cause mortality

9 of these pet. This study was conducted with the aimed to detect the hepatic problem in dogs

<sup>10</sup> and cats along with its frequency in different sex, age and breeds of animals. A total of 100

<sup>11</sup> clinically suspected animals (50 dogs and 50 cats) were included in the study. After thorough

<sup>12</sup> clinical examination, 2 ml bloods were collected by venue puncture in serum vials for

<sup>13</sup> biochemical analysis. After that all animals were subjected to x-ray and ultrasonography. In

 $_{14}$  biochemical analysis standard deviation of hepatic enzymes (902.48 u/l ALP, 626.66 u/l ALT,

<sup>15</sup> 722.98 u/l AST in dog and 564.22 u/l ALP, 300.06 u/l ALT, 340.00 u/l AST in cat) and

<sup>16</sup> bilirubin (2.02 mg/dl in dog and 5.22 mg/dl in cat) were remarkably above the mean value

17  $(333.86 \pm 127.63 \text{ u/l ALP}, 256.36 \pm 88.62 \text{ u/l ALT}, 261.62 \pm 102.24 \text{ u/l AST}, bilirubin 0.72 \pm 0.29$ 

 $mg/dl in dog and 245.42 \pm 79.79 u/l ALP, 180.16 \pm 42.43 u/l ALT, 188.99 \pm 48.08 u/l AST,$ 

19  $1.81\pm0.74$  mg/dl bilirubin in cat).

21 Index terms— serum biochemistry, x-ray, ultrasonography, hepatic problem, dogs and cats.

# <sup>22</sup> 1 Detection of Hepatic Problem in Dogs and Cats by Biochem <sup>23</sup> ical Assay and Imaging Technique (X-Ray and Ultrasonogra <sup>24</sup> phy)

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25 Introduction eeping pet has become a cultural phenomenon in the advanced world. Currently around 13 million of households (48%) own a pet (excluding fish) in the UK (Islam et al., 2013). Dogs (23%) and cats (18%) are 26 the most common pet animals in their houses in Britons currently. In the USA, around 62% of all households 27 have a pet (Pet Industry Market Size and Ownership Statistics, 2013). In terms of number of dogs and cats the 28 figures are around 78.2 million and 86.4 million respectively in the USA. A similar picture has been observed in 29 Europe, Australia, and South East Asia including China, Japan and India. In Bangladesh the pet populations are 30 also increasing day by day. Hypothetically, dog and cat populations in Bangladesh are predicted as 1.6 million 31 and 1 million respectively. This increasing trend of having a pet in the home has been attributed to various 32 reasons including 'misfiring of parental instincts, biophilia (a hypothetical biologically based love of nature), 33 social contagion, the tendency for the middle class to emulate the customs of the rich, the need to dominate 34 35 the natural world, social isolation in urban societies, and the desire to teach responsibility and kindness to 36 children' (Herzog, 2011). But several health problems of the pet make their owners more tensed and worried. 37 These health problems sometimes may cause mortality in pets. Hepatic problem is one of them. Infectious and inflammatory hepatobiliary diseases are common causes of morbidity and mortality in canine and feline patients 38 (Callahan et al., 2011). The prevalence of the hepatic problems in dog and cat is 1.24% and 0.41% respectively 39 (Apalkova, 2013). The common liver problems in dogs are hepatitis, chirrosis, bile duct obstruction, gall bladder 40 inflammation, circulatory or blood vessels abnormalities, cancer and toxins (Willard et al., 2012). In case of cat, 41 cholangiohepatitis, hepatic lipidosis and chronic hepatitis are the common problems of liver (Fluen et al., 2019). 42 Liver diseases are considered often to remain under diagnosed. Clinical signs can be absent for a long time until 43

the disease has progressed to a severe stage. Therefore laboratory findings, such as elevated liver enzymes in multiple samples, are what lead to suspect a liver disease more often than clinical signs.

Presence of the liver disease is then usually confirmed with more specific laboratory tests that give clue on liver function (Apalkova, 2013). Although serum biochemistry is considered as important preliminary tools for proceeding toward the correct diagnosis and treatment protocol, radiography is also useful to evaluate the morphologic abnormalities and ultrasonography (USG) is an excellent non-invasive way to evaluate liver

parenchyma (Kumar et al., 2012). The literature about biochemical alteration and imaging technique in liver

51 problems is scarce. As per our knowledge, there were no previous published reports on clinical finding and

- 52 laboratory alterations of liver disorders under Bangladeshi conditions. Hence, the present study was undertaken 53 to detect hepatic problems by assessing several aspects of blood biochemical profile and using imaging techniques
- (X-ray and USG) in clinically suspected animals. The study was basically focused on only hepatic problems
- <sup>55</sup> broadly not specific causes or condition in the liver.

#### 56 **2** II.

#### <sup>57</sup> 3 Materials and Methods

#### <sup>58</sup> 4 a) Ethical statement

The study was carried out with the permission of the director of clinics. The verbal consent from the animal's owner was taken as they visited to the clinics for treatment purpose of the animals. The sample was collected with minimum discomfort of the animal.

#### $_{62}$ 5 b) Study area, period and animals

<sup>63</sup> The present study was conducted through the period of October, 2020 to March, 2021 on 100 animals (50 dogs

and 50 cats) that were presented at Teaching and Training Pet Hospital and Research Center of Chattogram
Veterinary and Animal Sciences University. Animals of sexes (male and female), different age category like young

55 Veterinary and Animal Sciences University. Animals of sexes (male and female), different age category like young 66 (upto 1 year), adult (<1 to 5 years) and old (<5 years) and breeds were included in the study. The animals that

<sup>67</sup> had signs like anorexia, polyuria (PU), polydipsia (PD), abdominal distension, weight loss, anemia, and jaundice

were considered for sample collection.

#### <sup>69</sup> 6 c) Clinical examination

70 Each animal was subjected to a detailed clinical examination. Each animal was thoroughly evaluated for its

general condition, inspection of mucous membranes, hydration status, signs of pain, and abdominal distension.
Information regarding feeding history, polyuria, polydipsia and weight loss were known from the owner by face

73 to face interview.

#### 74 7 d) Blood and data collection

<sup>75</sup> Blood sample (2.5 ml) was collected from cephalic vein of each animal using sterile disposable needle. Before <sup>76</sup> collection, the puncture area was cleaned and disinfected with 70% alcohol. The collected blood was stored in <sup>77</sup> serum vials for biochemical analysis. Data regarding age, sex and breed were collected from hospital case record <sup>78</sup> sheet.

#### <sup>79</sup> 8 e) Serum biochemistry

After clotting, serum was separated by centrifugation and transferred to a dry clean vial for further evaluation. Humalyzer 3000 semi-automated chemistry system was used to determine the serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, total proteins, albumin, blood urea nitrogen (BUN), creatinine, cholesterol, and glucose. The hepatic problems were considered in those animals where the bilirubin, ALP, ALT and AST value were significantly above the reference value.

### <sup>85</sup> 9 f) Imaging technique (x-ray and ultrasonography)

All the animals were subjected to chest and abdominal x-ray. After x-ray exposure, abdominal ultrasonography was performed following appropriate procedure. Before USG scanning, the whole ventral abdomen was shaved in each animal. Abnormal morphology (altered size, shape and structure) in x-ray and parenchymal changes in ultrasonography were helped in diagnosis of hepatic problems in some animals.

#### <sup>90</sup> 10 g) Statistical analysis

All data were stored in excel sheet 2007 and descriptive statistics were calculated in its by using excel sheet
software. Frequency distributions were analyzed by using statistical software stata 2017. Significant was
considered when p-value<0.05 for chi square test. The occurrences were calculated by dividing total animals</li>

with positive case and multiplied by hundred.

95 **11 III.** 

#### 96 12 Results

#### <sup>97</sup> 13 a) Biochemical analysis findings

The standard deviation were found 2.02 mg/dl (bilirubin), 902.48 u/l (ALP), 626.66 u/l (ALT), 722.98 u/l (AST) 98 for dog and 5.22 mg/dl (bilirubin), 564.22 u/l (ALP), 300.06 u/l (ALT), 340.00 u/l (AST) for cat. The mean 99 values were 0.72±0.29 mg/dl (bilirubin), 333.86±127.63 u/l (ALP), 256.36±88.62 u/l (ALT), 261.62±102.24 u/l 100 (AST) for dog and 1.81±0.74 mg/dl (bilirubin), 245.42±79.79 u/l (ALP), 180.16±42.43 u/l (ALT), 188.99±48.08 101 u/l (AST) for cat. The higher standard deviation value for bilirubin, ALP, ALT and AST in both dogs and cats 102 indicates that the data above the mean value and were not clustered around the mean. The data showed that 103 there were remarkable changes on bilirubin, ALT, ALP and AST value that indicated hepatic problems in some 104 study animals (Table 3 and 4). 105

#### <sup>106</sup> 14 b) Imaging techniques (x-ray and ultrasonography) findings

In x-ray and ultrasonography, abnormal structural morphology (misshaped and enlarged liver), abdominal fluid and abnormal parenchymal lesion (hypoechoic foci and thickened capsule with hyperechoic foci) were found in some dogs and cats respectively (Figure ?? and 2).

### 10 15 c) Occurrence and frequency distribution of hepatic problem in dogs and cats

The occurrence of hepatic problems in dogs and cats were recorded 18% (9/50) and 12% (6/50) through 112 biochemical analysis respectively. In imaging techniques (x-ray and USG) it was found 6% (3/50) and 4%113 (2/50) hepatic problems in dogs and cats respectively. The occurrence of hepatic problems was higher in older 114 dogs (50%) and cats (50%) followed by young (0% in dog and 20% in cat) and adult (0% in dog and 5.13%115 in cat) which is statistically significant (P < 0.05) (Table 3 and 4). In case of sex, male dogs (22.22%) and 116 cats (20.0%) were more susceptible for hepatic problems than female (13.04% in dogs and 3.85% in cat) (Table 117 3 and 4). German shepherd dog breeds (42.86%) were more prone to hepatic problems followed by Lash apsu 118 (28.57%), Mixed breed (20.0%) and Local dogs (12.50%) in dogs (Table 3). Local cat breeds (19.05%) were highly 119 susceptible followed by Persian cat (10.53%), Mixed (0%) and Exotic cat (0%) for hepatic problems (Table ??). 120 The hepatic problems were considered in those animals where the bilirubin, ALP, ALT and AST value were 121 significantly above the reference value. Additionally abnormal morphology (altered size, shape and structure) in 122 x-ray and parenchymal changes in ultrasonography were also helped in some animals. 123 IV. 124

#### 125 **16** Discussion

Hepatic enzyme assay are performed widely as a popular measurement of the condition of the liver (Watson et al., 126 2009). These are alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). 127 Among these ALT and AST are present higher concentrations in cytosol of hepatocytes and elevated if there is 128 hepatocyte damage. The ALP and GGT enzymes are epithelial, membrane-bound produced from biliary tract in 129 response to certain stimuli, such as cholestasis (Watson et al., 2009). Increased level of these liver enzymes may 130 be a sign of damage to the liver (Center et al., 2007). In our study animal, standard deviation values (902.48 131 132 u/l ALP, 626.66 u/l ALT, 722.98 u/l AST in dog and 564.22 u/l ALP, 300.06 u/l ALT, 340.00 u/l AST in cat) of these enzymes were significantly larger than mean values (333.86±127.63 u/l ALP, 256.36±88.62 u/l ALT, 133 261.62±102.24 u/l AST in dog and 245.42±79.79 u/l ALP, 180.16±42.43 u/l ALT, 188.99±48.08 u/l AST in cat) 134 indicate remarkably elevation of these enzymes resulting from hepatic problems. These findings were supported 135 by the previous literature. Hyperbilirubinemia is found when the hepatic problem becomes severe stage (Watson 136 et al., 2009). As bilirubin may be normally found in the urine of dogs, very high levels suggest a liver disease. In 137 case of cats bilirubinuria is a clinically important finding (Watson et al., 2009). Standard deviations of bilirubin 138 (2.02 mg/dl in dog and 5.22 mg/dl in cat) were also higher than the mean value  $(0.72\pm0.29 \text{ mg/dl} \text{ in dog and } 100 \text{ mg/dl})$ 139  $1.81\pm0.74$  mg/dl in cat) in case of both dogs and cats that represents elevation in some animals. As there 140 were changes in the hepatic enzymes value, it might be related to the hepatobilliary problems in both dogs and 141 142 cats. Decreased albumin and total protein levels may be related to a liver disease, since liver is the only organ 143 producing albumin (Apalkova et al., 2013). Standard deviations of albumin and total protein were below the 144 mean values indicates data points were closed to the average in our study. Liver also metabolizes ammonia to urea, so blood urea nitrogen concentration may fall (Watson et al., 2009). Liver play role in metabolism, its 145 dysfunction may affect numerous other processes and thus change various laboratory findings, such as glucose 146 and cholesterol levels (Watson et al., 2009). Standard deviations value of albumin, total protein, BUN, glucose 147 and cholesterol were below the mean values indicate data points were closed to the average in our study. These 148 findings were not lined with the previous study. These variations may be due to initial stage of hepatic problems 149 or changes of these values were not significant in all hepatic problems diagnosed animal. 150

Abdominal ultrasonography (US) is the most useful technique for detecting a hepatobiliary disease (Guillot et al., 2009, Feeney et al., 2008). It shows the changes in the parenchymal structure as variation in echogenicity. This helps comparing hepatic tissue to other soft tissues, as well as determining heterogeneous structures within the hepatic parenchyma (Watson et al., 2009). Similarly, some parenchymal lesions like thickened hepatic capsule and hypoechoic lesion (cyst or tumor) in advance stage of hepatic problems were found in this study.

Radiography usually helps in liver diagnostics mostly to detect the size and shape of the liver and possibly 156 to point the lesions more specifically (Watson et al., 2009). Hepatomegaly may be seen as caudal dislocation of 157 gastric axis and pylorus in lateral view of the abdomen (Apalkova et al., 2013). Microhepatia is not as clearly 158 seen, but may be visible as a more perpendicular angle of gastric fundus to the spine in the right lateral projection 159 (Watson et al., 2009). Focal enlargement of one hepatic lobe is shown as dislocation of the organs nearest to the 160 lobe. Agreed with these statements there were some abnormal morphologies The occurrence of hepatic problems 161 in our study was recorded 18% and 12% in dogs and cats respectively in serum biochemistry. On the other 162 hand, 6% and 4% hepatic problems were recorded in dogs and cats respectively in imaging techniques (x-ray 163 and USG). These observations were so far higher than the observations of some studies where they recorded 164 1.24% and 0.41% in dogs and cats respectively (Apalkova et al., 2013) and 3.51% in dogs only (Bagherwal et al., 165 2018). Besides, our findings were also lower than a separate report on dogs (55.12%) conducted by Lakshmi et 166 167 al., (2017). This variation in prevalence may be due to study on clinically suspected animals, difference in sample 168 size and geographical variation. The figure in imaging techniques was lower and it may be due to morphological 169 and parenchymal changes occurs only when the hepatic problem becomes more severe and in advance stage.

Though all ages are susceptible for liver disease in dogs and cats, older animals (more than 5 years old) were more susceptible (Bagherwal et al., 2018). Similarly, the hepatic problems were higher (50% both in dogs and cats) in old animals (more than 5 years old) than young (0% and 20% in dogs and cats respectively) and adults (0% and 5.17% in dogs and cats respectively) in our study. Similar observations were found by Maindigers et al., **??**2004) where reported highest hepatic problems were in older animals aged more than five years or more. This may be due to mechanical use of the liver and also reduced in body self immunity with progression of age.

In sex wise measurements, males (22.22% and 20.83% in dogs and cats respectively) were highly susceptible for hepatic problems than female (13.04% and 3.85% in dogs and cats respectively) in our study. This observation was opposed with the observations of Bagherwal et al., (2018) where reported females were more susceptible than male. But supported with the findings of Lakshmi et al., (2017) where they found males were more prone to hepatic problems. This difference may be due to disproportionate sampling in the study animals.

181 Breed wise predisposition revealed German shepherd (42.86%) was highest proportion followed by Lash Apsu (28.57%), Mixed breed (20.0%) and Local dog (12.50%). This finding was not agreement with study of Bagherwal 182 et al., (2018) and Lakshmi et al., (2017) where they found higher proportion in Labrador Retriever followed 183 by German Shepherd, Cross breed, Rottweiler etc. Higher proportion in German shepherd may be due to 184 higher population of in the study area or disproportionate in sampling. In case of cat higher percentage of 185 hepatic problems were found in local cat (19.05%) followed by Persian cat (10.53%). Although there is no breed 186 predisposition for hepatic problems in cats (Apalkova et al., 2013), higher percentage in local cat in this study 187 may be due to increase tendency to visit outside that increase the chance of contact with other cats and dust. 188 ν. 189

#### <sup>190</sup> 17 Conclusion

Hepatic problems were highly found in clinical patients (dog and cat). Different breeds of male older aged animals were highly susceptible for hepatic problems in case of both dog and cat. Clinical history, clinical examination gave a clue of hepatic problems. Significantly altered hepatic enzymes made definitive diagnosis of hepatic problem as clinical signs become prominent in advance stage. Ultrasonography and x-ray were also helped to definitive diagnosis of hepatic problem in advance stage in some animals. According to our findings, it is highly suggestive to the pet owner to check their pets especially in older animal regularly to detect the hepatic problem in earlier and it will be helpful for effective treatment.

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<sup>&</sup>lt;sup>2</sup>Detection of Hepatic Problem in Dogs and Cats by Biochemical Assay and Imaging Technique (X-Ray and Ultrasonography)



Figure 1:

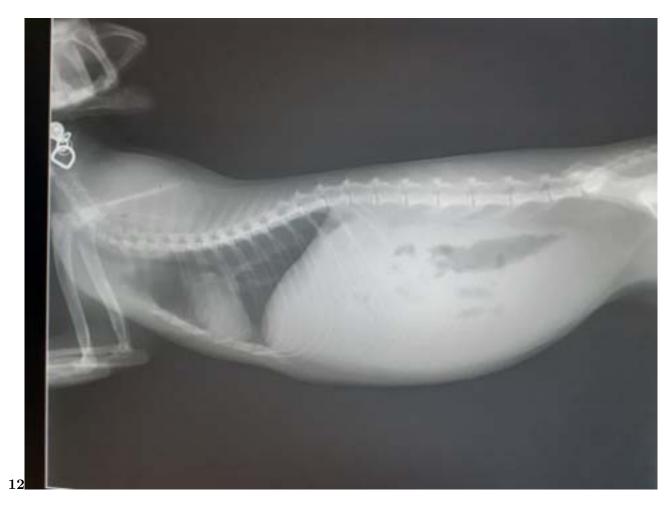


Figure 2: Figure 1 : Figure 2 :

1						
Parameters	Mean	Median	Standard devia- tion	MinimumMaximum Refe		Reference value
Bilirubin (mg/dl)	$0.72{\pm}0.29$	0.20	2.02	0.00	11.70	0.10-0.30
Total protein	$6.68 {\pm} 0.19$	6.50	1.32	4.00	10.20	5.40 - 7.50
(mg/dl)						
Albumin $(mg/dl)$	$2.91{\pm}0.10$	2.80	0.69	1.20	4.50	2.70 - 4.40
ALP (u/l)	$333.86{\pm}127.63$	61.35	902.48	10.70	5196.00	5.00 - 131.00
ALT $(u/l)$	$256.36{\pm}88.62$	67.45	626.66	18.50	3717.30	12.00 - 118.00
AST (u/l)	$261.62{\pm}102.24$	45.85	722.98	17.8	4154.40	15.00-66.00
Glucose (mg/dl)	$84.92 {\pm} 2.96$	84.60	20.97	37.5	130.80	70.00-138.00
Creatinine $(mg/dl)$	$1.60 {\pm} 0.26$	1.20	1.85	0.40	12.30	0.50 - 1.60
BUN (mg/dl)	$29.46 {\pm} 3.90$	20.17	27.58	10.30	154.40	6.00 - 25.00
Cholesterol (mg/dl)	$132.52{\pm}6.71$	120.00	47.43	59.00	280.60	92.00-324.00

Figure 3: Table 1 :

Parameters	Mean	Median	Standard devia- tion	Minimu	mMaximun	n Reference value
Bilirubin (mg/dl)	$1.81 {\pm} 0.74$	0.20	5.22	0.00	27.40	0.10-0.40
Total protein	$6.97 {\pm} 0.15$	6.90	1.09	4.90	9.20	5.20 - 8.80
(mg/dl)						
Albumin $(mg/dl)$	$2.82{\pm}0.09$	2.80	0.66	1.67	4.10	2.50 - 3.90
ALP (u/l)	$245.42{\pm}79.79$	34.60	564.22	7.80	2804.00	10.00-50.00
ALT (u/l)	$180.16{\pm}42.43$	45.65	300.06	12.30	1258.00	10.00-100.00
AST (u/l)	$188.99 {\pm} 48.08$	54.25	340.00	11.90	1704.40	10.00-100.00
Glucose $(mg/dl)$	$98.22 {\pm} 4.95$	87.40	35.02	45.60	232.60	50.00 - 170.00
Creatinine (mg/dl)	$1.32{\pm}0.15$	1.00	1.07	0.50	5.80	0.60 - 1.50
BUN (mg/dl)	$31.52{\pm}2.56$	27.10	18.12	12.60	110.40	14.00-36.00
Cholesterol (mg/dl)	$119.26{\pm}6.10$	104.00	43.11	67.00	218.60	75.00-220.00

Figure 4: Table 2 :

3

Explanatory variable	Co-variable	Total	+ve	Percentage	P value (? 2 test)
Age	Adult	25	0	0	0.00
	Old	18	9	50	
	Young	7	0	0	
Sex	Female	23	3	13.04	0.40
	Male	27	6	22.22	
Breed	German shepherd	7	3	42.86	0.53
	Labrador	2	0	0.0	

Figure 5: Table 3 :

#### $\mathbf{2}$

#### 17 CONCLUSION

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