

Liver biochemical and histopathological findings in dogs with experimentally induced exocrine pancreatic insufficiency

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Abstract

Routine liver biochemical parameters were evaluated in 8 dogs with exocrine pancreatic insufficiency (EPI) induced by surgical ligation of the pancreatic duct and the pancreatic branch of the pancreaticoduodenal artery and confirmed with the trypsin-like immunoreactivity test. Eight additional dogs were used as healthy controls. Data collection began at the 4th week postoperatively and continued weekly to the 21st week. In the dogs with EPI, the serum activity of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were consistently elevated. The serum total and conjugated bilirubin concentrations remained within normal limits throughout the experimental period. Histopathological study revealed hepatic lipidosis in the dogs with EPI. Therefore, since this condition seems to be an additional consequence of EPI in dogs, laboratory evaluation of dogs with EPI must include assessment of liver function, to determine if additional or different therapeutic measures are indicated.

Résumé

Les paramètres biochimiques hépatiques de routine ont été évalués chez 8 chiens avec insuffisance pancréatique exocrine (EPI) induite par ligature chirurgicale du canal pancréatique et de la branche pancréatique de l'artère pancréaticoduodénale et confirmée avec l'épreuve d'immunoréactivité à la «trypsine-like». Huit chiens supplémentaires ont servi de témoin. La collecte de données a débuté à la 4^e semaine post-chirurgie et a continué de façon hebdomadaire jusqu'à la 21^e semaine. Chez les chiens avec EPI, l'activité sérique de l'alanine aminotransférase, l'aspartate aminotransférase et la phosphatase alcaline étaient constamment élevées. Les concentrations de bilirubine sérique totale et de bilirubine conjuguée sont demeurées à l'intérieur des valeurs normales tout au long de la période expérimentale. L'examen histopathologique a révélé une lipidose hépatique chez les chiens avec EPI. Ainsi, comme cette condition semble être une conséquence additionnelle de l'EPI chez les chiens, une évaluation par analyses de laboratoire de chiens avec EPI doit inclure une évaluation de la fonction hépatique, afin de déterminer si des mesures thérapeutiques additionnelles ou différentes sont indiquées.

(Traduit par Docteur Serge Messier)

Canine exocrine pancreatic insufficiency (EPI) is a disease that leads to maldigestion and secondarily to malabsorption of nutrients. Bacterial overgrowth, destruction of the epithelium and reduction of the height of villi, infiltration of the duodenal lamina propria with lymphocytes and plasma cells, and changes in the activity of the jejunal brush-border enzymes are some possible consequences of EPI in the small intestine (1–3).

The consequences of canine EPI on liver structure and function are not well established. It has been reported that the serum activity of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) may occasionally be mildly or moderately elevated in untreated cases (2–4). Pancreatic fibrosis in humans was considered to be the cause of fatty infiltration, cirrhosis of the liver, or both, in a study of 1000 consecutive autopsies (5).

In dogs with experimentally induced EPI, we estimated the values of liver biochemical parameters that are routinely determined in everyday practice, as well as studying the livers histopathologically, to detect any effects of EPI on liver integrity. The study was part of a project (6) carried out with the approval of the State Veterinary Authorities.

We divided 16 intact, 8-mo-old mongrel dogs into 2 groups: 8 dogs in which EPI was experimentally induced and a control group of 8 dogs. In both groups, the sex ratio was 1:1. The dogs came from 4 litters of 4 dogs each. Before the experiment, they were housed in the wards at the Clinic of Companion Animal Medicine, School of Veterinary Medicine, Aristotle University of Thessaloniki, for 7 mo. They were routinely vaccinated and every 3 mo were given a parasiticide (Drontal plus; Bayer, Toronto, Ontario), 34.4 mg/kg. The

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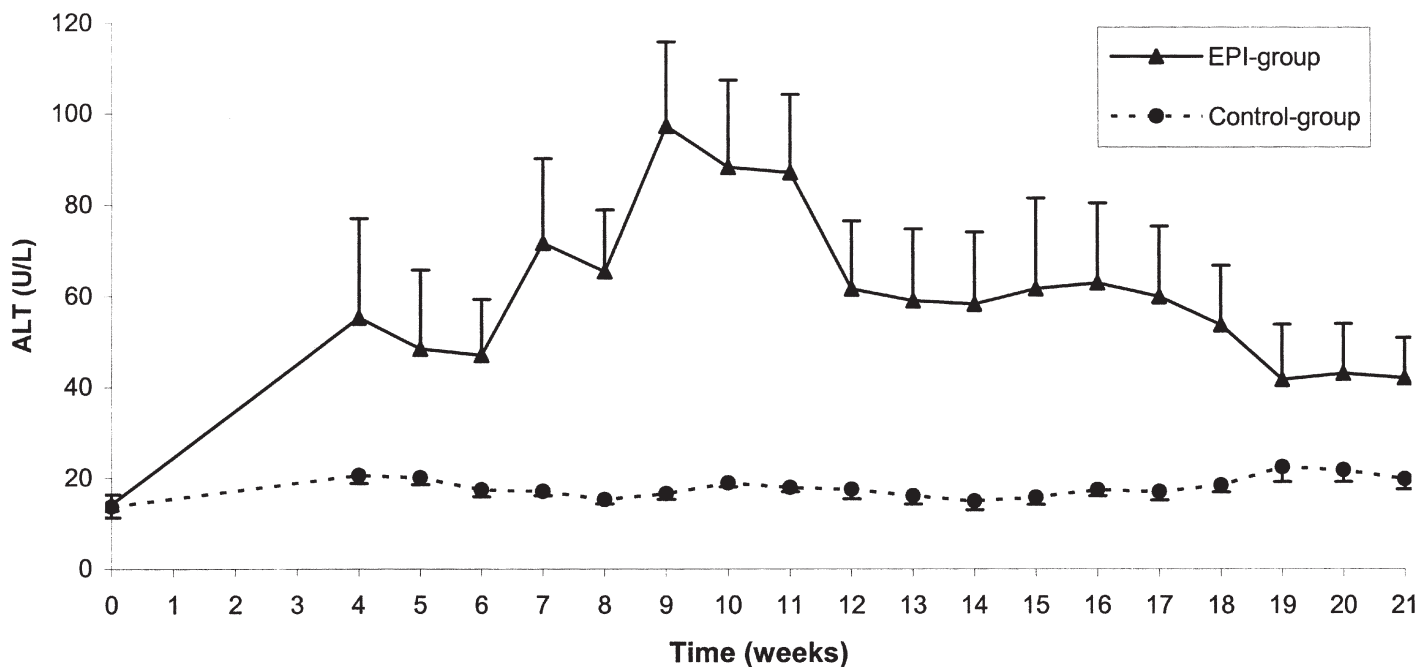


Figure 1. Serum alanine aminotransferase (ALT) activity [mean $\pm$ standard error (s_x)] in 8 dogs with experimentally induced exocrine pancreatic insufficiency (EPI) and 8 healthy control dogs. Time 0 is the preoperative period.

dogs were caged individually and given canned and dry food (Waltham and Friskies companies, respectively), in daily quantities determined from their body weight and activity (7). The diet consisted of 20% protein, 7% to 10% lipid, 1.5% to 3.0% fibre, 6% ash, and 9% water and supplied 500 to 1000 IU/g of vitamin A, 40 to 50 IU/g of vitamin D, and 5 to 10 IU/g of vitamin E.

For the surgical procedure, the dogs were given xylazine (Rombun; Bayer), 0.7 mg/kg, and atropine sulfate (Demo), 0.04 mg/kg, administered intramuscularly. Then general anesthesia was induced with thiopentone sodium (Pentothal; Abbott Laboratories, St-Laurent, Quebec), 10 mg/kg, administered intravenously, and maintained with a mixture of halothane (Fluothane; ICI Pharmaceuticals, Chennai, India) and oxygen in a semiclosed system. The pancreatic duct was ligated and transected through a ventral midline laparotomy. Additionally, the pancreatic branch of the pancreaticoduodenal artery was ligated and transected, and the pancreas was bluntly dissected from the duodenum. The integrity of the common bile duct was maintained. Postoperatively, the dogs were treated with a balanced electrolyte solution administered intravenously for 4 d.

In both groups, once preoperatively and every postoperative week from the 4th to the 21st, we determined the serum activity of ALT, aspartate aminotransferase (AST), and ALP, as well as the concentrations of total and conjugated bilirubin, using Boehringer Mannheim kits (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) and a Gilford spectrophotometer (Stasar II; Gilford Instruments Laboratories, Oberlin, Ohio, USA). At the same time, serum lipase and amylase activity and concentrations of glucose and total proteins were determined with Boehringer Mannheim kits and either a type 240 Gilford spectrophotometer (for serum lipase and amylase, which were assayed daily for the first 4 wk postoperatively and then weekly) or a Stasar II (for the others). In addition, the

hematocrit was determined by the Wintrobe method (8) and the complete blood count by the Unopette method (Becton Dickinson, Rutherford, New Jersey, USA). On the same schedule, urine samples were collected from both groups by cystocentesis; the specific gravity was measured with a handheld refractometer (American Optical, New York, New York, USA), and the pH and concentrations of proteins, glucose, ketones, bilirubin, blood, and hemoglobin were determined with the Boehringer Mannheim Combur test.

A test for trypsin-like immunoreactivity (TLI) (TLI Unit, Royal Veterinary College, London, England) was performed in blood collected from the EPI dogs after 12 h of fasting at the end of the 17th wk.

At the end of the experiment (21st wk), all the dogs in both groups were euthanized and underwent necropsy immediately. Samples of pancreas, liver, kidney, duodenum, and myocardium were fixed in 10% formalin in buffered saline. Paraffin-embedded sections were stained with hematoxylin and eosin, Gomori's stain, the periodic acid-Schiff reaction, and Sudan red. Liver specimens were evaluated without knowledge of the group to which the animal belonged and subjectively graded as 0 (no histopathological evidence of lipidosis), + (a minority of hepatocytes contained lipid), ++ (about 50% of the hepatocytes contained lipid), or +++ (the majority of the hepatocytes contained lipid).

Statistical analyses were performed separately for each parameter using a PROC MIXED statistical analysis system (SAS, version 8; SAS, Cary, North Carolina, USA) (9). The case or control status of the animal was the between-subjects factor and the time since the operation the within-subjects factor. In this repeated-measures factorial experimental design, the main interest was in how the mean values of the parameters of the differently treated animals changed over time and how the treatment differences changed over time. Initially, models assuming different covariance structures — namely,

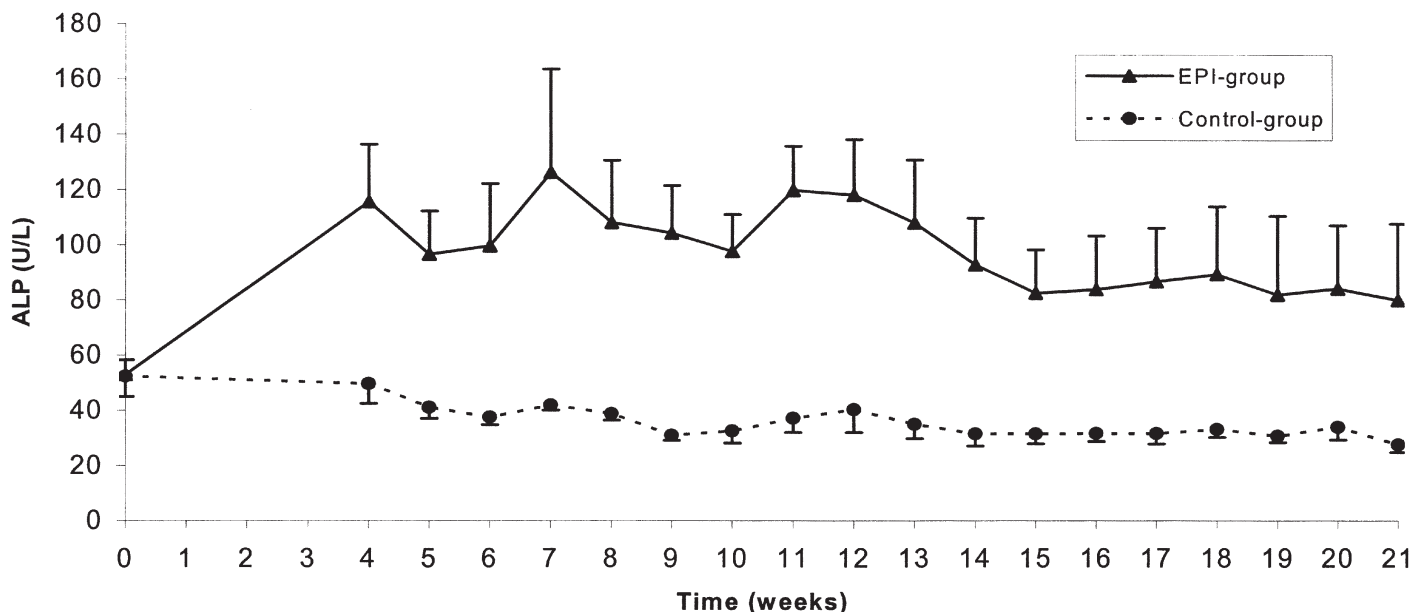


Figure 2. Serum alkaline phosphatase (ALP) activity (mean $\pm$ standard error [s_x]) in the same 2 groups of dogs. Time 0: before the beginning of the experiment (preoperatively); EPI-group: dogs with exocrine pancreatic insufficiency; Control-group: healthy dogs.

compound symmetry, first-order autoregressive, and unstructured — were fitted to the observed data. The decision on which of the 3 structures provided the best fit was based on the smallest Akaike's Information Criterion. Subsequently, the values of each parameter were modelled as polynomial functions of time since the operation. All the statistical results were evaluated for significance at the 5% level.

Postoperatively, clinical signs of acute pancreatitis (vomiting, diarrhea, depression, and anorexia) developed in all the EPI dogs for 3.5 d. This condition was confirmed by a striking increase in serum lipase and amylase activity for 15 d postoperatively. The serum glucose and total protein determinations, as well as the hematologic examination and the urinalysis, did not reveal any abnormalities. Approximately 4 wk after surgery, the EPI dogs showed ravenous appetite, pica, coprophagy, diarrhea, gross and microscopic steatorrhea, gradual weight loss, growth retardation, and seborrhea sicca, which worsened thereafter. In the EPI dogs, there was a significant quadratic ($P < 0.001$, $P = 0.007$) and cubic ($P = 0.02$, $P = 0.001$) effect of postoperative time on the activity of ALT and ALP, respectively. The regression equations that best fitted the observed data were $ALT = -8.9 (14.1) + 26.7 (5.8) \text{ time} - 2.4 (0.6) \text{ time}^2 + 0.06 (0.02) \text{ time}^3$ (Figure 1) and $ALP = 27.04 (19.1) + 31.2 (7.6) \text{ time} - 3.1 (0.9) \text{ time}^2 - 0.08 (0.03) \text{ time}^3$ (Figure 2), where the standard errors of the coefficients are in parenthesis. There was no effect of time on postoperative AST or bilirubin values in either group, but the AST activity was higher in the EPI dogs (Figure 3) and the total and conjugated bilirubin concentrations were higher in the control dogs throughout the observation period ($P < 0.001$). The values of all laboratory parameters were within normal limits in the control dogs throughout the experiment, as well as in the EPI dogs preoperatively. The TLI values in the EPI dogs were lower than $3 \mu\text{g/L}$ (reference range 5 to $35 \mu\text{g/L}$).

In the EPI dogs, the pancreas was firmer than normal and extremely small, the liver was hyperemic, and the small intestine was dilated, with pulpy contents, mucosal erosions, and ulcers. No such abnormalities were found in the control dogs. Atrophy and fibrosis of the pancreas, destruction of the epithelium of the villi, with reduction of their height, and infiltration of the duodenal lamina propria by lymphocytes and plasma cells were seen in the EPI dogs. In this group, the liver specimens revealed hepatic lipodosis (HL) and large (intervascular) droplets. In all the EPI dogs, large number of the hepatocytes containing lipid appeared empty, with an inconspicuous nucleus. The HL was graded as + in 2 dogs and ++ in the other 6. There were no histopathological lesions in the kidneys or myocardium of the EPI dogs or in any of the sampled tissues of the control dogs.

The ALT, ALP, and AST activity in the EPI dogs was elevated throughout the experimental period, suggesting that this is a consistent finding in dogs with experimentally induced EPI. Alarline aminotransferase is a hepatocyte-specific enzyme. An increase in its serum activity is principally due to hepatic parenchymal disease, whereas only minor increases are expected with damage to erythrocytes or striated muscle cells. On the other hand, the moderate increase in AST activity may be attributable to the shorter half-life of AST than of ALT (12 versus 48 h) or to the fact that AST is found in the hepatocyte mitochondria and is liberated only when hepatocytes are destroyed, thus reflecting more serious hepatic disease (1). Although AST is not considered a liver-specific enzyme, since it can also be liberated in significant quantities from other tissues (erythrocytes and muscle cells), the simultaneous increase in ALT and AST activity suggests a hepatic origin. Alkaline phosphatase is thought to be useful in evaluating biliary disease in dogs. Hepatobiliary or bone disease, hyperadrenocorticism, and glucocorticoid or anticonvulsant administration can cause increased ALP values (10). In the EPI dogs, since other causes

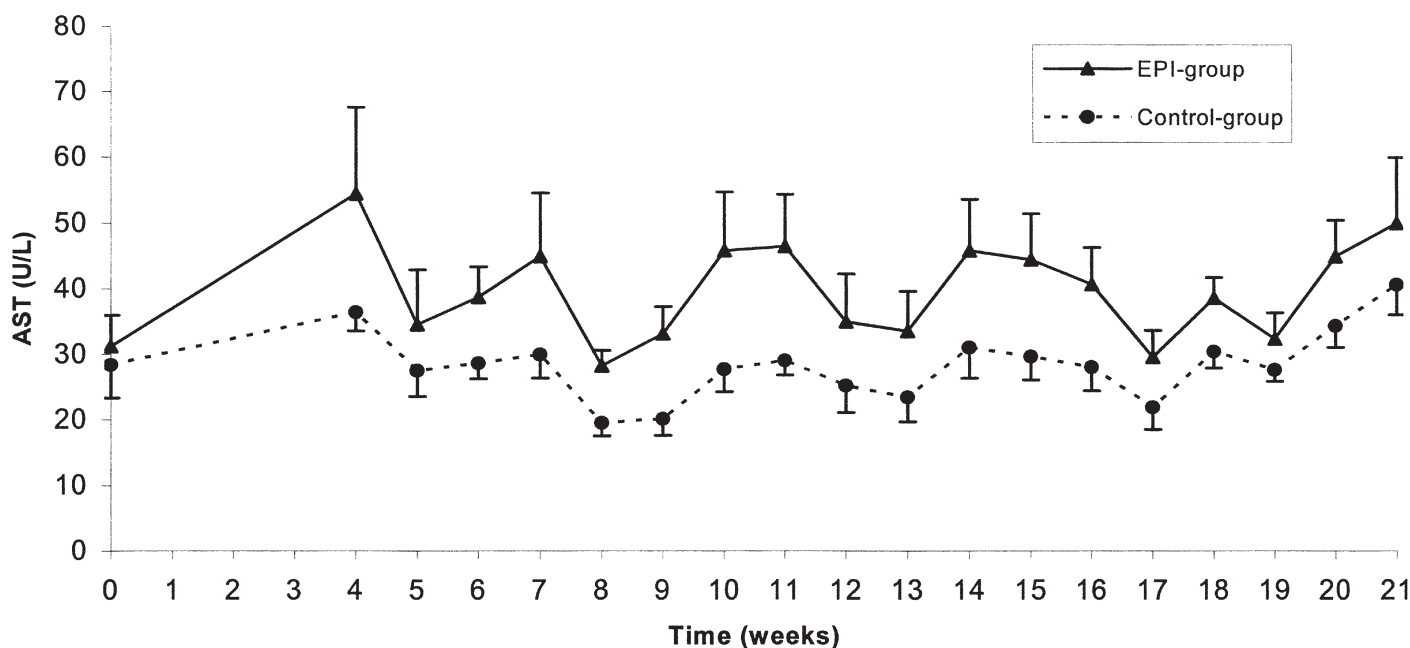


Figure 3. Serum aspartate aminotransferase (AST) activity (mean $\pm$ standard error [s_x]) in the same 2 groups of dogs. Time 0: before the beginning of the experiment (preoperatively); Exocrine pancreatic insufficiency (EPI)- group: dogs with exocrine pancreatic insufficiency; Control-group: healthy dogs.

could be excluded, hepatopathy was thought to be the main reason for the increased ALT, AST, and ALP activity.

Bilirubin concentrations were lower in the EPI dogs throughout the experimental period. However, the values were not lower than the lowest normal reference values. There was no evidence of hypoproliferative anemia, malignant neoplasia, or end-stage renal disease, the main causes of hypobilirubinemia (11). Since hepatic involvement was suspected in the EPI dogs, bilirubin concentrations might have been raised (1,11). However, there must be considerable hepatocellular disease for hyperbilirubinemia to occur, because the liver reserve capacity for bilirubin processing is up to 30 times the normal bilirubin load (1). Therefore, the serum bilirubin concentration is not a sensitive indicator of hepatocellular function (12), increasing only in severe hepatocellular disease (1).

The serum albumin concentration is usually measured when severe liver insufficiency is suspected (13). However, this determination might have been of no value in our study, because EPI, which is associated with maldigestion and malabsorption syndromes (3), is considered to cause hypoalbuminemia (13).

Laboratory tests are often essential to identify hepatic disease, assess its severity, and monitor its progression (1,14). However, there are no laboratory tests identifying a specific problem. Thus, once biochemical tests indicate that hepatic disease exists, the diagnosis must be made histopathologically (1,12,14,15). The final diagnosis of mild to moderate HL in the EPI dogs in our study was based on the histopathological findings; fat-specific stains were used to exclude vacuolar hepatopathy.

Hepatic lipidosis, an excessive accumulation of fat (triglycerides and phospholipids) in the hepatocytes, can be caused by nutritional, metabolic, hormonal, toxic, or liver hypoxic disturbances (14,16). Some etiologic factors, such as protein-calorie malnutrition, protein

deficiency, starvation, small intestinal bacterial overgrowth, and bacterial antigens taken up from the intestine, can be related to EPI (16,17). Choline deficiency has been extensively studied in the pathogenesis of HL in humans (16). In dogs with EPI, HL may be due to a deficiency of choline and other lipotropic substances resulting from the incomplete digestion and absorption of dietary protein in the absence of pancreatic proteolytic enzymes (5). Furthermore, decreased apoprotein synthesis is also the result of reduced protein absorption. Apoprotein is necessary for the conversion of triglycerides to lipoproteins, the only form in which lipid is excreted from the liver (16,18). Moreover, EPI may cause deranged macromolecular transport (16). In our study, these abnormalities may have contributed to the production of HL in the EPI dogs. This condition was confirmed histologically in dogs after ligation of the pancreatic ducts (19) and in humans with pancreatic fibrosis and subsequent EPI (5).

In the EPI dogs, no other conditions associated with HL, such as prolonged overnutrition, obesity, toxins, hypoxia, and diabetes mellitus (17), were present. Diabetes mellitus must be considered in dogs with EPI (3). In our subjects, no true evidence was apparent from the weekly serum glucose determinations.

Laboratory abnormalities in humans with HL are typically mild. Elevated serum levels of transaminase, γ -glutamyltransferase, ALP, and bilirubin are the most common. The degree of the biochemical disturbances does not correlate with the extent or severity of the HL (16). Clinicopathological evidence of the disorder in dogs is marked by increased activity of liver enzymes, particularly ALP (17).

The control dogs were not subjected to a sham operation, because manipulation of abdominal organs would have been justified only if an acute condition were being studied. The clinical and laboratory signs of EPI appeared approximately 4 wk after surgery and worsened thereafter. However, the symptoms of acute pancreatitis and

Table 1. Mean $\pm$ standard error (s_x) of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities (U/L), and total (TB) and conjugated (CB) bilirubin concentrations (mg/dL) in healthy dogs and in dogs with experimentally induced exocrine pancreatic insufficiency (EPI)

Parameter weeks	ALT			ALP			AST			TB			CB		
	EPI-group	Controls		EPI-group	Controls		EPI-group	Controls		EPI-group	Controls		EPI-group	Controls	
0	14.2 $\pm$ 2.2	13.7 $\pm$ 2.4		53 $\pm$ 5.2	52.4 $\pm$ 7.4		31.2 $\pm$ 4.7	28.4 $\pm$ 5.1		0.13 $\pm$ 0.02	0.13 $\pm$ 0.02		0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	
4	55.2 $\pm$ 21.9	20.6 $\pm$ 1.8		115.5 $\pm$ 20.7	49.6 $\pm$ 7.2		54.4 $\pm$ 13.2	36.4 $\pm$ 2.9		0.13 $\pm$ 0.02	0.12 $\pm$ 0.01		0.09 $\pm$ 0.02	0.08 $\pm$ 0.01	
5	48.4 $\pm$ 17.3	20.1 $\pm$ 1.5		96.5 $\pm$ 15.6	41 $\pm$ 4		34.5 $\pm$ 8.4	27.5 $\pm$ 4		0.14 $\pm$ 0.02	0.15 $\pm$ 0.01		0.06 $\pm$ 0.01	0.09 $\pm$ 0.01	
6	47 $\pm$ 12.3	17.4 $\pm$ 1.5		99.5 $\pm$ 22.5	37.4 $\pm$ 2.7		38.7 $\pm$ 4.6	28.6 $\pm$ 2.4		0.11 $\pm$ 0.01	0.12 $\pm$ 0.01		0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	
7	71.6 $\pm$ 18.5	17.1 $\pm$ 0.9		126 $\pm$ 37.3	41.9 $\pm$ 1.9		44.9 $\pm$ 9.6	29.9 $\pm$ 3.6		0.13 $\pm$ 0.03	0.12 $\pm$ 0.02		0.07 $\pm$ 0.01	0.08 $\pm$ 0.01	
8	65.4 $\pm$ 13.5	15.2 $\pm$ 0.9		108 $\pm$ 22.5	38.7 $\pm$ 2.5		28.2 $\pm$ 2.4	19.5 $\pm$ 2		0.13 $\pm$ 0.01	0.14 $\pm$ 0.02		0.08 $\pm$ 0.01	0.09 $\pm$ 0.01	
9	97.2 $\pm$ 18.6	16.6 $\pm$ 1.3		104.2 $\pm$ 17.1	30.9 $\pm$ 1.9		33.1 $\pm$ 4.1	20.1 $\pm$ 2.5		0.14 $\pm$ 0.01	0.15 $\pm$ 0.01		0.07 $\pm$ 0.01	0.1 $\pm$ 0.01	
10	88.2 $\pm$ 19	18.9 $\pm$ 0.9		97.6 $\pm$ 13.2	32.5 $\pm$ 4.4		45.8 $\pm$ 8.9	27.7 $\pm$ 3.5		0.13 $\pm$ 0.01	0.14 $\pm$ 0.01		0.08 $\pm$ 0.01	0.07 $\pm$ 0.01	
11	87 $\pm$ 17.1	17.9 $\pm$ 0.9		119.6 $\pm$ 15.8	37.1 $\pm$ 5.2		46.4 $\pm$ 7.9	29 $\pm$ 2.2		0.15 $\pm$ 0.03	0.14 $\pm$ 0.01		0.09 $\pm$ 0.02	0.07 $\pm$ 0.01	
12	61.5 $\pm$ 15	17.5 $\pm$ 2.1		117.9 $\pm$ 20	40.2 $\pm$ 8.3		34.9 $\pm$ 7.3	25.2 $\pm$ 4.1		0.1 $\pm$ 0.01	0.14 $\pm$ 0.01		0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	
13	59 $\pm$ 15.7	16.1 $\pm$ 1.9		107.9 $\pm$ 22.6	34.9 $\pm$ 5.2		33.5 $\pm$ 6	23.4 $\pm$ 3.8		0.12 $\pm$ 0.01	0.15 $\pm$ 0.02		0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	
14	58.2 $\pm$ 15.8	14.9 $\pm$ 1.9		92.7 $\pm$ 16.9	31.5 $\pm$ 4.5		45.8 $\pm$ 7.8	31 $\pm$ 4.7		0.13 $\pm$ 0.01	0.15 $\pm$ 0.01		0.07 $\pm$ 0.01	0.1 $\pm$ 0.002	
15	61.6 $\pm$ 19.9	15.7 $\pm$ 1.5		82.4 $\pm$ 15.7	31.4 $\pm$ 3.6		44.4 $\pm$ 6.9	29.6 $\pm$ 3.5		0.14 $\pm$ 0.01	0.19 $\pm$ 0.01		0.08 $\pm$ 0.01	0.1 $\pm$ 0.01	
16	62.9 $\pm$ 17.5	17.4 $\pm$ 1.4		83.7 $\pm$ 19.4	31.6 $\pm$ 3		40.6 $\pm$ 5.6	28 $\pm$ 3.6		0.12 $\pm$ 0.01	0.16 $\pm$ 0.02		0.06 $\pm$ 0.01	0.09 $\pm$ 0.01	
17	59.9 $\pm$ 15.4	17 $\pm$ 1.8		86.6 $\pm$ 19.4	31.6 $\pm$ 3.9		29.5 $\pm$ 4.1	21.9 $\pm$ 3.5		0.13 $\pm$ 0.01	0.13 $\pm$ 0.02		0.08 $\pm$ 0.01	0.09 $\pm$ 0.02	
18	53.6 $\pm$ 13.1	18.4 $\pm$ 1.5		89.2 $\pm$ 24.5	33.1 $\pm$ 3		38.5 $\pm$ 3.2	30.4 $\pm$ 2.6		0.11 $\pm$ 0.01	0.16 $\pm$ 0.02		0.06 $\pm$ 0.004	0.09 $\pm$ 0.01	
19	41.6 $\pm$ 12	22.4 $\pm$ 3.3		81.9 $\pm$ 28.4	30.6 $\pm$ 2.2		32.3 $\pm$ 4	27.6 $\pm$ 1.7		0.11 $\pm$ 0.01	0.16 $\pm$ 0.03		0.07 $\pm$ 0.01	0.08 $\pm$ 0.01	
20	43 $\pm$ 10.9	21.7 $\pm$ 2.6		84.1 $\pm$ 22.8	33.9 $\pm$ 4.7		44.9 $\pm$ 5.5	34.3 $\pm$ 3.3		0.12 $\pm$ 0.01	0.14 $\pm$ 0.01		0.07 $\pm$ 0.01	0.09 $\pm$ 0.01	
21	42 $\pm$ 8.8	19.7 $\pm$ 2.2		79.9 $\pm$ 27.6	27.5 $\pm$ 2.7		49.9 $\pm$ 10	40.6 $\pm$ 4.6		0.12 $\pm$ 0.01	0.11 $\pm$ 0.005		0.06 $\pm$ 0.01	0.08 $\pm$ 0.01	

the elevated activity of serum lipase and amylase were apparent in the EPI dogs for 3.5 and 15 d postoperatively, respectively.

Our data show that ALT, AST, and ALP activity was consistently elevated in the dogs with untreated EPI and that this was due to the development of HL. Therefore, laboratory evaluation of dogs with EPI must include the assessment of liver parameters, in order to determine whether the liver is involved and whether additional or different therapeutic measures need to be instituted.

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