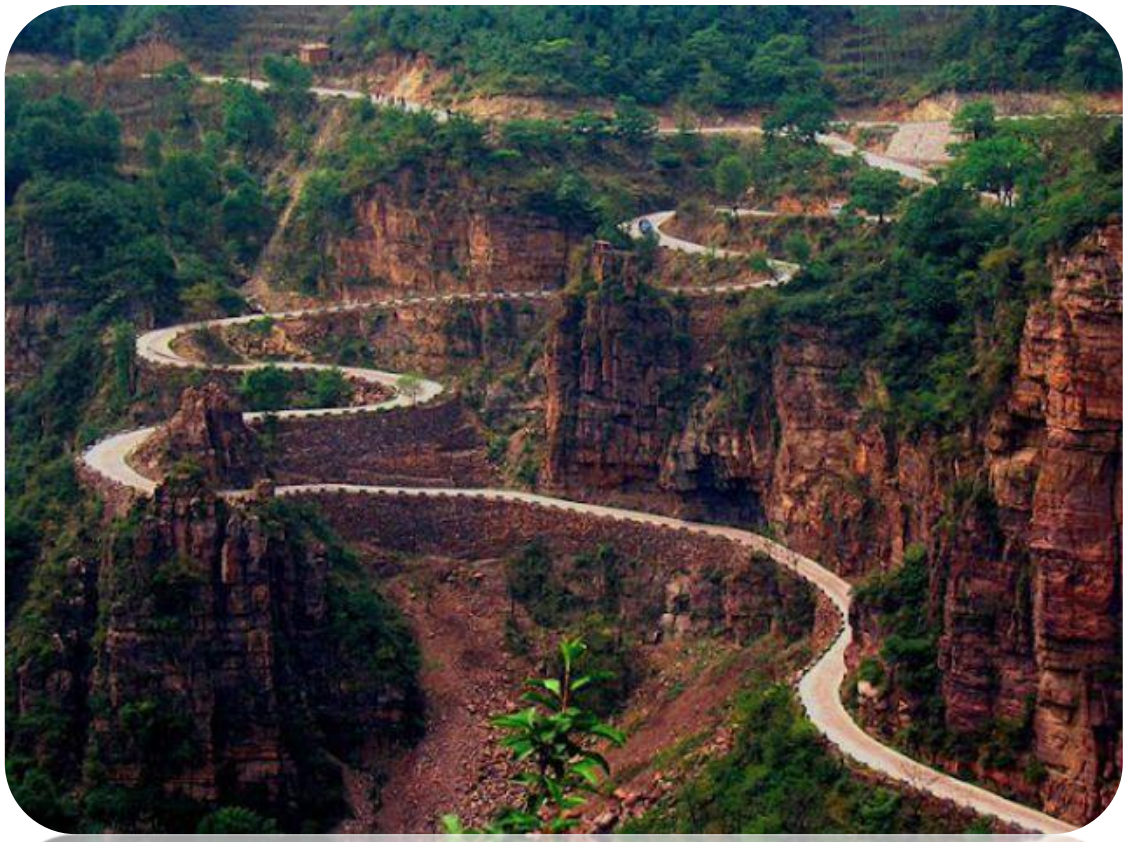
A Retrospective Study of Feline Hepatic Lipidosis in the Netherlands: 2003- 2010



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June 2014

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Abstract

Feline hepatic lipidosis (FHL) is characterized by lipid accumulation in hepatocytes causing impairment of liver functions and is potentially fatal. Characteristics of the Dutch population of cats with hepatic lipidosis concerning clinical findings, laboratory profile, clinical outcome and survival were evaluated and are described in this report. Cases were included by an established diagnosis based on cytological and/or histopathological findings. All 44 cats included were referred to the department of Clinical Sciences of Companion Animals of Utrecht University between 2003 to 2010. Patient files were searched for relevant data and a telephonic questionnaire was set up to contact the owners or referring veterinarians for follow up concerning clinical outcome and survival. Differences in survival rates for sex, age and severity of lipidosis were evaluated and variables were screened for prognostic value. Most cats were middle-aged and more female than male cats were affected. Lethargy, weight loss and anorexia were most repeated clinical signs. Less commonly repeated were jaundice and neurological signs. No bleeding tendencies were noted. Blood examination revealed frequent anemia, elevated enzyme activities of ALAT, GGT and AP, hyponatremia, hypokalemia, hypocalcemia and hypoalbuminemia with apparent normal total protein. Survival analysis showed that most cats died within the first month after initial diagnosis and that hypoalbuminemia significantly decreased survival time. No significant differences in survival time were detected for sex, age or severity of lipidosis.

Feline Hepatic Lipidosis

Feline hepatic lipidosis (FHL) is a common and potentially fatal liver disorder. FHL is characterized by an accumulation of triacylglyceraldehydes (TAG) in vacuoles in the hepatocyte cytosol. Accumulation of TAG will lead to impaired hepatocyte function, cholestasis, liver failure and eventually to death if not resolved. Although a common feline liver disorder, the overall prevalence of FHL is difficult to determine since many cats develop FHL secondary to any disease that will impair nutrient uptake[1]. Studies in North America done in primary care practices indicate that overall prevalence in feline patients ranges from 0.06% to 0.16%[2]. Cats with FHL are usually middle aged and often obese with the owners frequently mentioning a weight loss of ≥25%. In addition, the incidence of FHL seems to be higher in female cats compared to male cats[1]. The syndrome can occur as a primary or secondary form. The primary or idiopathic form can be caused by inadequate intake during periods of forced excessive and rapid weight loss (i.e. crash diet), unintentional food deprivation, change to a food unacceptable to the cat, sudden change in lifestyle or stress. Secondary hepatic lipidosis is caused by periods of anorexia instigated by an underlying disease such as pancreatitis, inflammatory bowel disease or other major organ dysfunction[2]. A similar condition in humans is nonalcoholic fatty liver disease (NAFLD) and is also mainly characterized by hepatic steatosis with obesity[3,4].

The development of anorexia in a cat causes the mobilization of TAG stored in adipose tissue due to a hormonal shift from a high to low insulin/glucagon ratio, which stimulates the hormone sensitive lipase (HSL). HSL is an enzyme that resides in adipose cells which catalyzes hydrolysis of TAG under influence of several hormones such as glucagon, (nor)adrenalin and adrenocorticotrophic hormone, HSL catalyzes hydrolysis of TAG in adipose tissue. The non-esterified fatty acids (NEFA's) that are produced are transported into the bloodstream and are taken up by the liver[5,6]. When transported into the hepatocytes, NEFA's can follow two pathways. One is transportation into mitochondria and breakdown via the β-oxidation process. The other pathway is (re-)assimilation into TAG in the cytosol. In cats with FHL the majority of NEFA's follow the latter pathway and generate TAG, which is stored in vacuoles in the cell. Consequently, these vacuoles increase in size until they eventually interfere with normal hepatic function[7]. Different explanations have been proposed for the accumulation of TAG in vacuoles. One explanation is a deficiency in L-carnitine, an amino acid that is required for the transportation of fatty acids into the mitochondria. Another is a possible lack of apolipoprotein B100 that is used in secretion of very low-density lipoproteins (VLDL's). However, none of these two theories are supported by conclusive evidence[2]. An overall explanation seems to be that cats have difficulties adapting their protein en lipid metabolism to a reduced intake of nutrients. Cats are depended on several essential amino and fatty acids and seem incapable of reducing the use of these elements[8].

Major clinical signs of FHL are a prolonged period of anorexia or reduced appetite, weight loss, lethargy, vomiting and weakness. Jaundice is predominantly seen in severe FHL (>70%) and hepatomegaly can often be palpated[1]. Neurological signs are uncommon but when electrolyte imbalances are severe enough (hypokalemia, hypophosphatemia), signs as head or neck ventroflexion and ptyalism may occur. Hypokalemia has also been reported to be associated with a shortened survival time[1]. However, hypokalemia, hypophosphatemia and hypomagnesemia occur in less than 30% of cats with FHL[9]. Total bilirubine, enzyme activities of alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are usually increased, with total bilirubin and AP being the most consistent in FHL cats. An increase in activity of gamma-

glutamyltranspeptidase (GGT) (twofold or greater) is uncommon and indicative of a underlying necroinflammatory disease such cholangitis[10]. A disturbed coagulation profile has been reported in less than 50% of cats with FHL, for which vitamin K depletion, with reduced intake, is the most likely explanation[11].

Diagnosis of FHL is based on clinical history, clinical signs, physical examination, laboratory work and ultrasound with cytological and/or histological confirmation. Currently, the golden standard for the diagnosis of FHL is histological examination by liver biopsy. However this method has major disadvantages like a high risk of bleeding due to a disturbed coagulation profile and the fragile tissue characteristics, slow tissue repair as a result of the catabolic state and anesthetic risk. Fine needle aspirate biopsy (FNAB) has limited tissue damage, and usually there is no need for anesthethics[9,12]. FHL and vacuolar hepatopathy in general, are accurately detectable with FNAB and cytological examination, although a possible (focal) underlying disease may be missed[13]. However, considering the risks, fine needle aspiration biopsy and following cytological examination is the preferred course of action to confirm FHL. Although research in North America has been done to assess the characteristics of the FHL population[1], there is little known about FHL patients in the Netherlands. The purpose of this study is twofold: first, to describe the characteristics of the Dutch population of FHL patients including clinical, laboratory and pathological findings and second, to evaluate clinical outcome and prognostic factors for a shortened survival time.

Methods and materials

Patients were selected from the records of the Veterinary Pathology Diagnostic Centre and of the University Veterinary Diagnostic Laboratory with the histopathological and cytological findings confirming the presence of hepatic lipidosis. Forty-four cases with an established diagnosis of hepatic lipidosis were included in the study. Diagnosis was based on histopathological findings by biopsy (ultrasound-guided Tru Cut or wedge biopsies), post mortem examination and cytology performed by fine needle aspiration biopsy. All included cases were referred between 2003 and 2010 to the department of Clinical Sciences of Companion Animals, University of Utrecht (CSCAUU), the Netherlands. All forms of lipidosis (slight, moderate and severe) were included independent of diagnosis of concurrent disease. Patient files were digitally stored by commercial veterinary software (Vetware®) and searched for relevant data concerning signalment, clinical history, findings on physical examination, laboratory results, ultrasound findings, cytology and histology results, and clinical outcome. A telephonic questionnaire was set up to contact the owners for follow up including clinical outcome concerning remission, recurrence or persistence of clinical signs related to FHL, and moment and the presumable cause of death. Data from the questionnaire was collected in May 2014. Statistical analysis was performed by use of commercially available software packages (IBM SPSS Statistics version 20, IBM Corporation and GraphPad Prism version 5, Graphpad Software, Inc.). The Kolmogorov-Smirnov test in combination with histogram plot were used to asses normality of all data. Survival fractions were calculated with the Kaplan-Meier method and survival time was defined as the period of time between initial cytological or histopathological diagnosis and date of death. Cats that died of FHL related causes were counted as events. Unrelated deaths, cats lost to follow up and cats that were still alive were censored. Significant differences between Kaplan-meier curves were tested by a Log Rank (Mantel-Cox) test. Prognostic factors for shortened survival time were identified by Cox regression models.

Results

Signalment

Forty-four cats were eligible for inclusion in this study and comprised 0,48% of the total referred population of cats from 2003-2010. Twenty-five (57%) were female (24 castrated) and 19 (43%) were male (18 castrated). Median (range) age of cats diagnosed with FHL was 9 years (0,5-18). Twenty-nine (66%) cats were middle aged (5-12 years) with 5 (11%) cats being younger than 5 years of age and 10 (23%) cats being older than 12 years. Median (range) bodyweight of cats was 4 (2-7) kg. Of the 44 cats 28 (64%) were European Shorthair, 4 (9%) were British Shorthair, 3 (7%) were Persian and 2 (5%) were Siamese. One of each were Burmese, Devon Rex, Main Coon, Norwegian forest cat, Oriental Shorthair, Ragdoll and Russian Blue.

Clinical history

In the history most mentioned complaints were lethargy (61%), weight loss (61%) and anorexia (52%). Less mentioned signs were reduced appetite (34%), vomiting (34%) and jaundice (14%) (Table 1). Reduced appetite was defined as reduced but not an absence of nutrient intake. Seventeen (39%) cats had concurrent diseases reported at the time of initial examination. The median (range) for days of reduced appetite and anorexia were 10 (7-42) and 5 (1-35) days respectively (Table1). Reduced appetite was less common than anorexia although it often preceded anorexia. Considering the graver impact of anorexia, reduced appetite prior to anorexia was excluded from the reduced appetite variable. Signs of stress prior to clinical signs were mentioned in 5 cases (moving to a new house, new cats in the household and absence of the owner for a prolonged period). Neurological signs were uncommon: one cat showed signs of head tremors, one cat showed signs of changed behavior (no recognition of owner, yawning, tics) and one cat had neurological signs related to a concurrent chemotherapy.

Table 1 Clinical sign, duration in days of reduced appetite and anorexia and percentage of bodyweight loss at initial examination (n=44, 2003-2010).

	n
Lethargy	27/44
Weight loss	27/44
Anorexia	23/44
Reduced appetite	15/44
Vomiting	15/44
Stress	6/44
Jaundice	5/44
Diarrhea	4/44
Neurological signs	3/44
Ptyalism	2/44

	Median	Range
Anorexia (days)	5	1-35
Reduced appetite (days)	10	7-42
Loss of bodyweight (%)	21	3-48

Clinical examination

Findings during physical examination at initial diagnosis are shown in Table 2. The body condition of 15 cats was scored (BCS) and it revealed that 12 (80%) had a score of 2 or less out of 5 and were considered underweight or very thin. Only one cat had an ideal BCS and 2 cats were given a score of 5 out of 5 indicating obesity. Twenty-nine (66%) cats were diagnosed with a concurrent disease. The most common diseases were cholangitis (18%), pancreatitis (11%), neoplasia (9%) and diabetes mellitus (9%). Less frequent concurrent diseases were hyperthyroidism, diseases categorized as respiratory, cardiovascular, gastrointestinal and urogenital. Least frequent were amyloidosis, peritonitis and dermal lesions (Table 3). Twenty-five cats (57%) were considered lethargic or soporous. Tachypnea occurred in 14 of 35 (40%) cats and tachycardia was reported in 16 of 38 (42%) cats. Hypothermia (<38.5 °C) occurred in 30 of 39 (77%) cats with most (64%) cats having an body temperature between 37 and 38.5 °C. Normothermia (38.5-39 °C) occurred in 4 of 39 (10%) cats and hyperthermia or fever occurred in 5 of (13%) cats. The mucus membranes of 11 of 32 (34%) cats were icteric. Three of 18 (17%) cats showed signs of pain during abdominal palpation. An enlarged liver was palpable in 3 of 13 cats (23%). No bleeding tendencies were noticed in any of the cats.

Table 2 Body condition score (BCS) and findings during physical examination at initial diagnosis.

BCS (5 point scale)	n
(1) Very thin	4/15
(2) Underweight	8/15
(3) Ideal	1/15
(4) Overweight	0/15
(5) Obese	2/15

		n	%
Concurrent disease		29/44	66
Lethargy		25/44	57
Tachypnea		14/35	40
Tachycardia		16/38	42
Hypothermia		30/39	77
Jaundice		11/32	34
Abdominal palpation	Painfull abdomen	3/18	17
	Enlarged liver	3/13	23

Table 3 Concurrent diseases in cats with FHL (n=44, 2003-2010)

	Frequency	% cats
Cholangitis	8	18
Pancreatitis	5	11
Neoplasie	4	9
Diabetes mellitus	4	9
Hyperthyreodie	3	7
Respiratory	3	7
Cardiovascular	3	7
Gastrointestinal	2	5
Urogenital	2	5
Amyloidosis	1	2
Peritonitis	1	2
Dermal lesions	1	2

Hematology

The results for complete blood counts (CBC's) and coagulation profile of cats with FHL are given in table 4 and figure 1 and 2. Data was not normally distributed and therefore preference was given for median over mean. Complete blood counts were not performed in all cats due to selection of analyses per case. Although the median for prothrombin time (PT) was within reference limits, 7 of 18 (39%) cats showed an increase in PT. Nine of 18 (50%) cats showed an increase in APTT. One cat reported an PT and APTT value above measurement level and was considered an outlier and is not shown in figure 1. Fibrinogen was decreased (<1g/L) in 5 of 18 (28%) cats and elevated in 6 of 18 (33%) cats. Most cats showed normal thrombocyte counts, only 1 of 12 (8%) cats had a count below reference limit. Overall 9 of 18 (50%) cats had one or more abnormalities in their coagulation profile. Packed cell volume (PCV) was low in 13 of 29 (45%) cats although the median was within reference limits. Leucocytosis was detected in 5 of 28 (18%) cats. Lymphocyte count was low in 19 of 28 (68%) cats. Ten of 28 (36%) cats showed a high count of segmented neutrophils and 15 of 28 (54%) had a low count of eosinophils.

Table 4 Hematology results in cats with FHL.

Parameter	n	Median (Range)	Reference interval	Decreased	Increased
PT (sec)	18	13.1 (10.5-80.0)	10,5-16,3	0/18	7/18
APTT (sec)	18	37.9 (12.4-180.0)	10-25	0/18	9/18
Fibrinogen (g/L)	18	1,3 (0.3-7.2)	1-2	5/18	6/18
Platelet (10^9/L)	12	340 (155-735)	156-626	1/12	1/12
MCV (fl)	11	43 (29.5-52.0)	37.0-55.0	3/11	0/11
MCHC (mmol/L)	11	20.7 (19.4-22.7)	16.3-22.3	0/11	1/11
MCH (fmol)	11	,88 (0.61-1.05)	0.71-1.07	1/11	0/11
PCV (L/L)	29	0.28 (0.18-0.43)	0.28-0.47	13/29	0/29
WBC (10^9/L)	28	12.9 (3.6-27.4)	6,3-19,6	5/28	5/28
Lymphocyt (10^9/L)	28	1,4 (0.3-8.9)	2.0-7.2	19/28	1/28
Monocyt (10^9/L)	28	,35 (0-1.2)	0.1-1.0	3/28	1/28
Juvenile (10^9/L)	27	,0 (0-0)	<0.0	NA	0/27

Banded neutrophil (10^9/L)	27	,0 (0-1.9)	0.0-0.1	NA	1/27
Segmented neutrophil (10^9/L)	28	10,7 (1.4-19.2)	3.0-13.4	3/28	10/28
Eosinophil (10^9/L)	28	,2 (0-3.3)	0.3-1.7	15/28	2/28
Basophil (10^9/L)	28	,0 (0-0.3)	0.0-0.1	NA	1/28
Normoblast (10^9/L)	26	,0 (0-1.0)		25 (=0)	1 (=1)

NA=not applicable, High = number of values above upper reference limit, Low = number of values below lower reference limit. Each value represents one cat. PT = prothrombin time, APTT = Activated partial tromboplastin time, MCV = mean cellular volume, MCHC = mean cellular haemoglobin concentration, MCH = mean cellular haemoglobin, PCV = packed cell volume, WBC = White blood cell count.

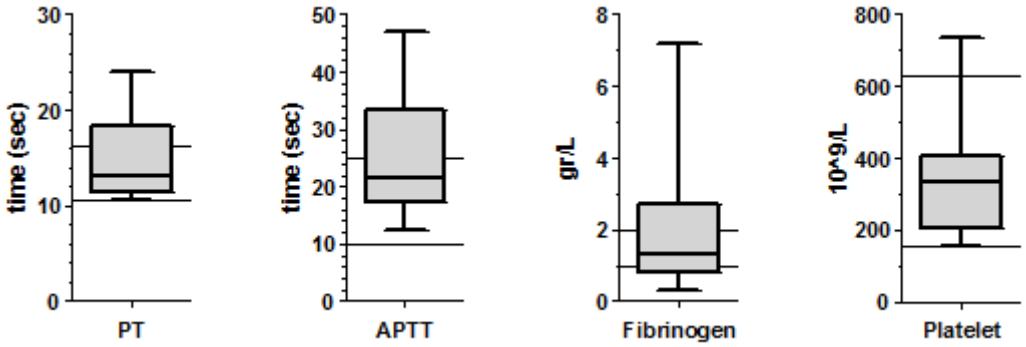

Figure 1 Coagulation profile showed no large deviations from the reference interval. In 18 FHL cats protrombin time (PT), activated partial tromboplastin time (APTT) and the concentration of fibrinogen were measured. Platelet count was determined in 12 cats. Whiskers represent 5 and 95th percentile. The horizontal lines indicate the lower and upper reference interval limits.

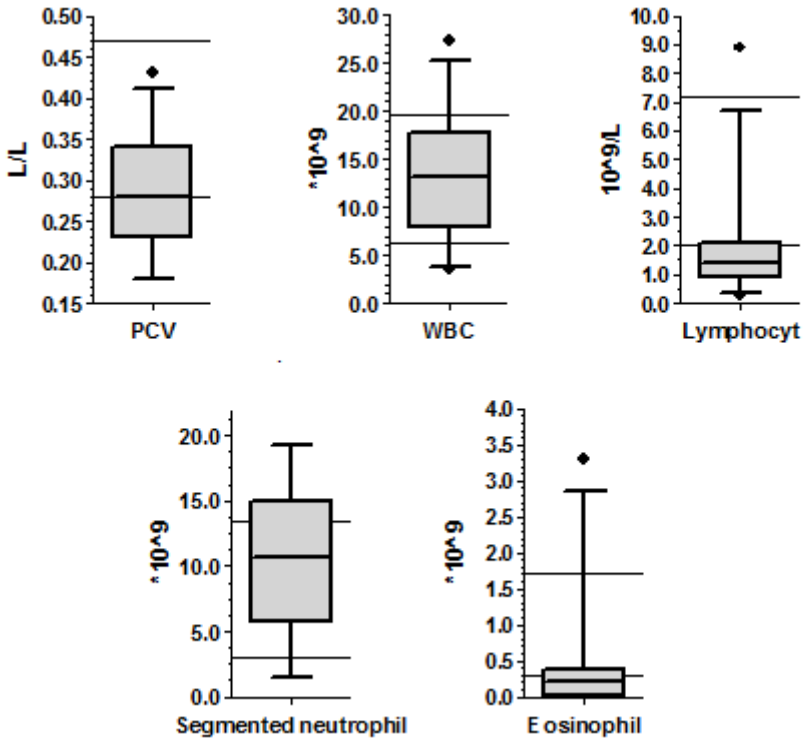

Figure 2 Slight changes in blood cell counts in FHL cats. Patients frequently showed a decreased packed cell volume (PCV, n=29), decreased lymphocyte counts (n=28) and eosinophil counts (n=28). Neutrophil counts (n=28) were slightly elevated. Whiskers represent 5 and 95th percentile. The horizontal lines indicate the lower and upper reference interval limits.

Clinical chemistry

The results of the clinical chemistry are shown in table 5 and figures 3,4 and 5. Bile acids were elevated in 23 of 32 (72%) cats with a median almost three-fold of the upper reference interval limit. Elevated alanine aminotransferase (ALAT) activity was observed in 17 of 23 (74%) cats with a two-fold increased median. Gamma glutamyl transferase (GGT) and alkaline phosphatase (AP) were increased in 3 of 5 (60%) and 6 of 8 (75%) cats, respectively. Electrolytes were frequently low although only calcium had a median lower than the lower reference interval limit. Sodium was low in 14 of 29 (48%) cats, potassium in 13 of 29 (45%) cats and calcium in 10 of 18 (56%) cats. Phosphate was decreased in 5 of 19 (26%) cats. Chlorine was elevated in 10 of 17 (59%) cats with a median slightly higher than the upper reference interval limit. Nine of 33 (27%) cats were hypoproteinemic and 12 of 32 (38%) cats were hypoalbuminemic. Hyperproteinemia occurred in 7 of 33 (21%) cats. Globulin profile varied with alpha 2 and gamma globulins showing the most frequent abnormalities. Three of 21 (14%) cats had an increase in alpha 1 globulins while alpha 2 globulins were increased in 17 of 21 (81%) cats. Beta 1 and beta 2 globulins were low in 8 of 21 (38%) cats while gamma globulins were elevated in 14 of 21 (67%) cats. Ten of 28 (36%) cats showed a low urea concentration while 9 (32%) had an increased urea concentration. Creatinine also had a wide range similar to urea but 8 of 30 (26.7%) of cats had an high concentration and five cats (17%) showed a low concentration. Glucose was elevated in 22 of 22 (100%) cats measured with a median two-fold above reference interval. Of these cats only 4 had a history of diabetes mellitus on initial examination. Fructosamine was measured in 10 of these cats and showed that 9 (90%) had increased values. Thyroxine was elevated in 2 of 9 (22%) cats. The concentration of feline pancreatic lipase (fPLI) was elevated in 4 of 5 (80%) cats in which it was analyzed. Lastly, none of the cats (n=13) in which a FeLV/FIV SNAPtest was performed tested positive for either FIV or FeLV.

Table 5 Clinical chemistry results in cats with FHL.

Parameter	n	Median (range)	Reference range	Decreased	Increased
Glucose (mmol/L)	22	13.6 (5.8-22.9)	3,4-5,7	0/22	22/22
Fructosamine (µmol/L)	10	393 (191-861)	156-240	0/10	9/10
Urea (mmol/L)	28	8.1 (3.3-67.5)	6,1-12,8	10/28	9/28
Creatinin (µmol/L)	30	118 (44-330)	76-164	5/30	8/30
Bileacids (µmol/L)	32	33.5 (0-215)	1-13	1/32	23/32
ALAT (U/L)	23	151 (22-521)	30-73	1/23	17/23
ASAT (U/L)	2	102 (84-120)	*No reference*	NA	NA
GGT (U/L)	5	6 (0-8)	<5	NA	3/5

AP (U/L)	8	85 (14-587)	12-52	0/8		6/8	
Sodium (mmol/L)	29	147 (128-157)	146-158	14/29		0/29	
Potassium (mmol/L)	29	3.4 (2.4-4.3)	3,4-5,2	13/29		0/29	
Calcium (mmol/L)	18	2.28 (0.82-2.80)	2,36-2,86[1]	10/18		0/18	
Phosphate (mmol/L)	19	1.20 (0.38-3.78)	0,89-2,05	5/19		3/19	
Chlorine (mmol/L)	17	113 (92-127)	105-112	5/17		10/17	
Magnesium (mmol/L)	4	0.99 (0.54-1.39)	*No reference*	NA		NA	
Total protein (g/L)	33	61 (31-86)	54-70	9/33		7/33	
Albumin (g/L)	32	26 (14-39)	25-34	12/32		3/32	
α1 (g/L)	21	4 (2-10)	2-5	0/21		3/21	
α2 (g/L)	21	9 (4-15)	4-7	0/21		17/21	
β1 (g/L)	21	4 (2-7)	4-7	8/21		0/21	
β2 (g/L)	21	4 (2-9)	4-9	8/21		0/21	
γ (g/L)	21	10 (5-35)	4-8	0/21		14/21	
Thyroxine (nmol/L)	9	18 (2-114)	15-45	4/9		2/9	
fPLI (µg/L)	5	21,0 (0.9-53.8)	<3,5	0/5		4/5	
FIV (SNAPtest)	13	NA	NA	neg	13/13	pos	0/13
FeLV (SNAPtest	13	NA	NA	neg	13/13	pos	0/13

NA=not applicable, High = number of values above upper reference limit, Low = number of values below lower reference limit. Each value represents one cat. ALT = Alanine transaminase, AST = aspartate transaminase, GGT = Gamma-glutamyl transferase, AP = alkaline phosphatase, fPLI = feline pancreatic lipase immunoreactivity. FIV and FeLV are results by SNAPtests that were performed by referring veterinarians (within one month of diagnosis) or by the university veterinary diagnostic laboratory (UVDL). [1]This reference range is for adult cats (≥1 year), of the one cat younger than 1 year no phosphate was determined.

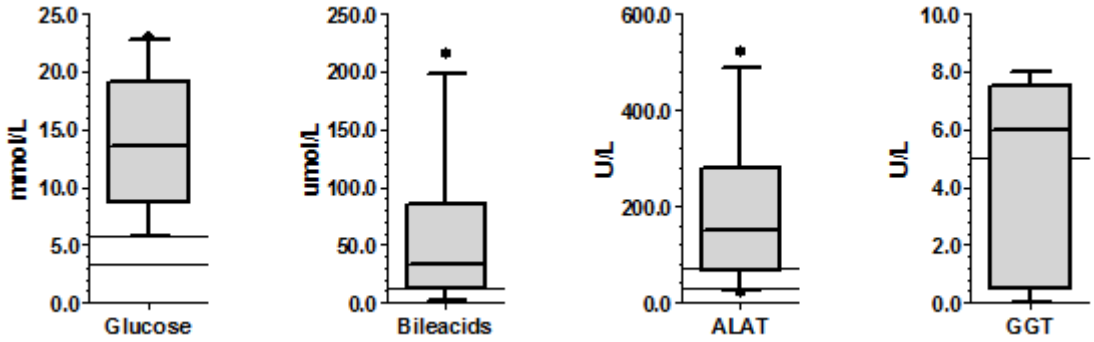

Figure 3 Aberrant variables in clinical chemistry in FHL cats. Glucose concentrations were all above reference interval (n=22). Bile acids (n=32) and enzyme activities of alanine transaminase activity (ALAT, n=23) and Gamma-glutamyl transferase (GGT, n=5) were also frequently elevated. Whiskers represent 5 and 95th percentile. The horizontal lines indicate the lower and upper reference limits.

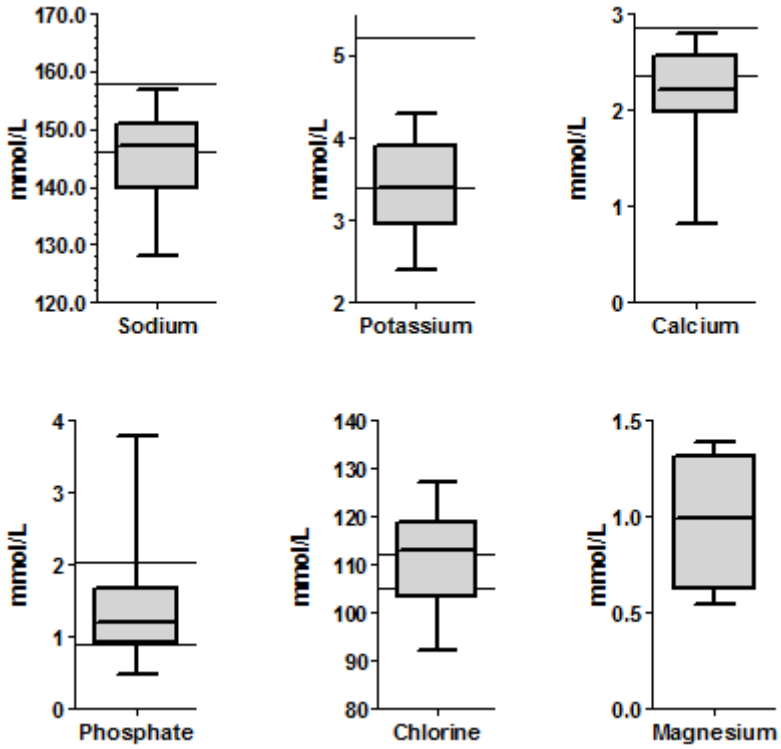

Figure 4 Distributions of electrolyte concentrations in FHL cats. Sodium (n=29), potassium (n=29) and calcium (n=18) concentrations were frequently low. Phosphate (n=19) levels were usually within reference interval limits and chlorine (n=17) concentrations were frequently elevated. Magnesium concentrations were measured in 4 cats. Whiskers represent 5 and 95th percentile. The horizontal lines indicate the lower and upper reference interval limits with the exception of magnesium since no reference interval limits for cats are known.

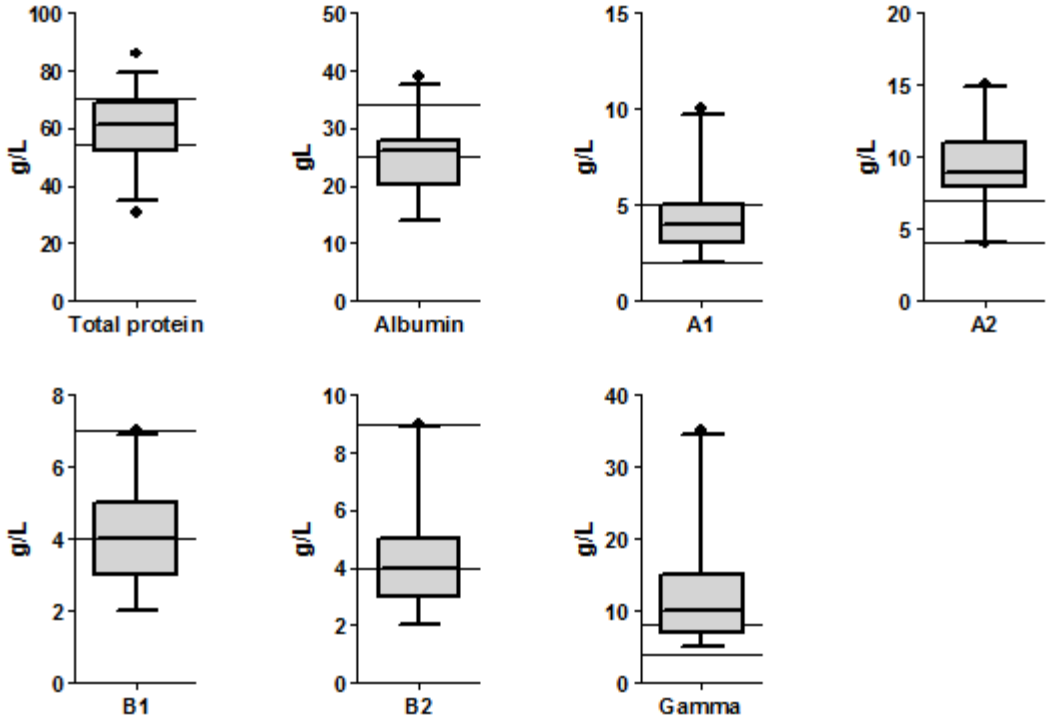

Figure 5 Serum protein profile in FHL cats. Total protein levels (n=33) were predominantly within reference interval limits with albumin concentrations (n=32) frequently decreased. Protein spectrum in FHL cats (n=21) frequently showed an increase in α2 and γ proteins with normal α1 levels and decreased β1 and β2 concentrations. Whiskers represent 5 and 95th percentile. The horizontal lines indicate the lower and upper reference interval limits.

Ultrasonographic examination

The results of ultrasonic examination of 42 cats with FHL are shown in table 6. Twenty-two (52%) cats showed an enlarged liver. Hyperechoic liverstructure was reported in 22 cats (52%). In 7 (17%) cats abnormalities in the gallbladder or ducts were seen. Pancreatic abnormalities were reported in 7 (17%) cats. Abdominal effusion was seen in 3 cats (7%) and 1 cat was diagnosed with an extrahepatic portosystemic shunt. In 10 (24%) cats abnormalities in the kidneys were detected ranging from irregular small kidneys to an enlarged renal pelvis.

Table 6 Ultrasonographic examination findings of cats with FHL (n=42, 2003-2010)

		n	%
Enlarged liver		22	52
Focal lesions		3	7
Parenchyma	hyperechoic	22	52
	hypoechoic	3	7
	cysts	1	2
	irregular	1	2
	patch like appearance	3	7
Abnormalities in the biliary system		7	17
Vascular anomalies		1	2
Abdominal effusion		3	7
Pancreas		7	17
Other abnormalities	Stomach	3	7
	Intestines	2	5
	Kidneys	10	24
	Spleen	2	5

Cytological and histopathological examination

In total, 33 cats were diagnosed with liver lipidosis by cytological examination alone, 6 cats by histopathological examination and 5 by a combination of both methods (table 7). Histopathological examination was performed on US Tru Cut biopsies (n=10) or necropsy (n=1). Thirty-eight cats (86%) in total underwent FNAB. Fourteen of 38 (37%) cats were classified as slight lipidosis, 18 of 38 (47%) cats as moderate lipidosis, and 5 (13%) cats as severe lipidosis. One cat showed no lipidosis on the first examination but had a slight lipidosis on the subsequent cytology report. Another cat showed a moderate hepatic lipidosis at first and a slight on the second cytological examination. Eleven cats were examined by histopathology, 6/11 were classified as having slight lipidosis and 5 having moderate lipidosis. Three of the 5 cats examined by both methods had similar results. One cat diagnosed as having no lipidosis by cytology was reported to have a slight lipidosis at histopathology. Another cat was classified by cytology as having moderate lipidosis and slight lipidosis at histopathology. In both cases biopsies were taken within 24 hours after FNAB. Concurrent processes were reported in 7 cats. Four reported signs of inflammation, 2 cats showed neoplasia and one reported amyloid accumulation.

Table 7 Cytological and histopathological results (n=44, 2003-2010).

	No lipidosis	Slight	Moderate	Severe	Total
Cytology	1	14	18	5	38
Histology		6	5		11

	Neoplasia	Inflammation	Amyloidosis
Cytology	1	2	1
Histology	1	2	
Cats	2	4	1

Cytology n = 33, Histopathology n = 6, both methods n = 5.

Clinical outcome and Survival

Clinical outcome and survival time (months) after diagnosis for the different severities of FHL are shown in table 8. In 17 cats clinical signs went into remission of which 4 cats had a recurrence of FHL signs. Twenty-three cats had persistent signs of FHL until death (either FHL related or not). Thirty-seven cats in total died, 4 cats were lost to follow up and 3 cats were still alive at the time of follow-up. Twenty cats died with signs of FHL prior to death (i.e. lethargy, anorexia or reduced appetite and weight loss) determined by the clinical report or by owner or referring veterinarian and were marked as events (i.e. FHL related death). Seventeen cats died due to reasons unrelated to FHL (e.g. extreme respiratory distress, pain caused by urogenital problems, euthanasia after diagnosis neoplasia) (figure 6). Estimated median survival time could only be calculated for severe lipidosis. Survival curves of FHL are shown in Figure 6, 7, 8, and 9. Significant differences between the curves were calculated by a Log Rank (Mantel-Cox) test and demonstrated no significant differences in survival time for any of the curves.

Table 8 Clinical outcome after diagnosis of FHL at follow-up (n=44, 2003-2010).

	Feline hepatic lipidosis			
	Slight	Moderate	Severe	Total
No. of patients with follow up	16/18	19/21	5/5	40/44
Clinical outcome				
Remission (recurrence)	9 (2)	7 (2)	1 (0)	17(4)
Persistent	7	12	4	23
Alive at follow up	2	1	0	3
Death (FHL related)	14 (5)	18 (11)	5 (4)	37 (20)
Survival time (months)				
EMST (95% CI)	N/A	N/A	0.1 (0-0.207)	N/A

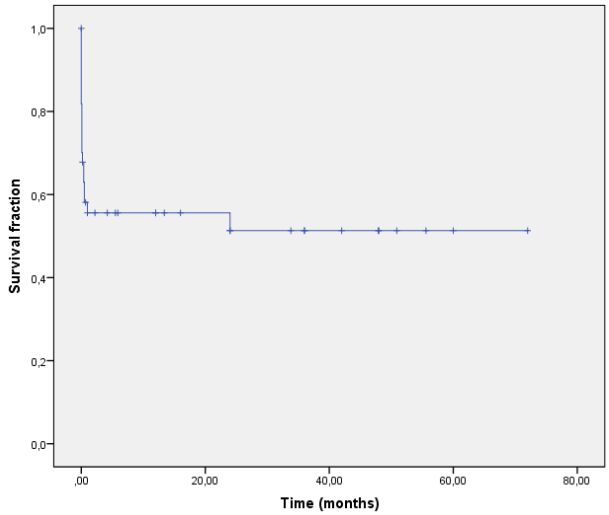

Figure 6 Kaplan-Meier curve for the survival time (months) for FHL cats (n=44; 2003-2010). Notice the steep decline in survival fraction of FHL cats within the first month after initial diagnosis. Twenty-four cases were censored (vertical bars) due to a different cause of death (n=17), lost to follow-up (n=4) or they were still alive (n=3).

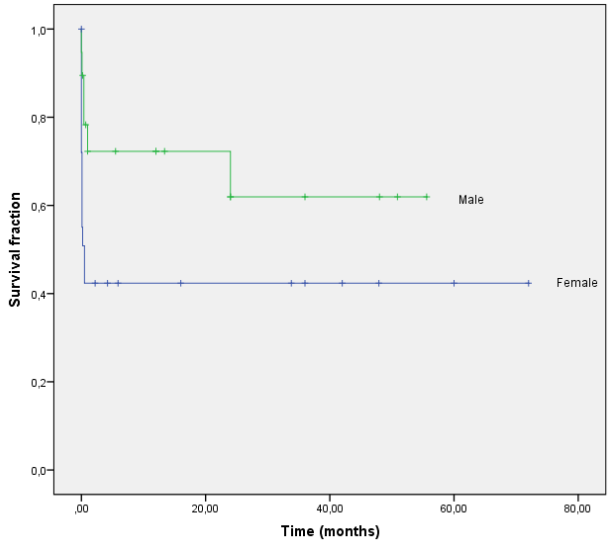

Figure 7 Kaplan-Meier curves for survival time (months) of male (n=19) compared to female (n=25) cats after diagnosis with FHL (n=44; 2003-2010). Female cats appear to have a smaller survival fraction compared to male cats. Censored cases are indicated by vertical bars. A Log Rank (Mantel-Cox) showed no significant difference between the curves (p=0.063).

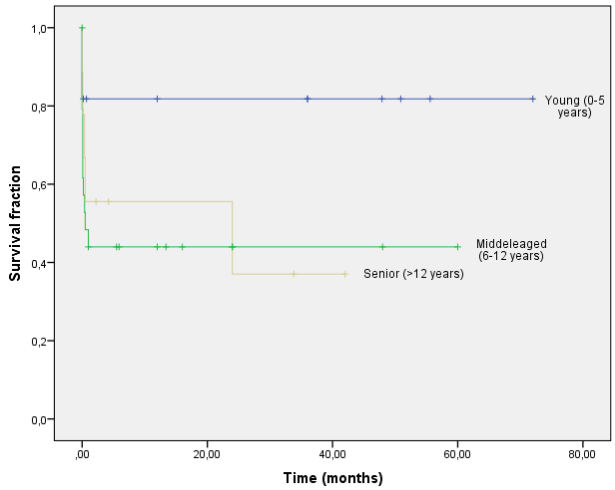

Figure 8 Kaplan-Meier curve for the survival time (months) for different age groups in cats diagnosed with FHL (n=44; 2003-2010). Cats were grouped in young (0-5 years, n=11), middle-aged (6-12 years, n=24) and senior (>12 years, n=9). Censored cases are indicated by vertical bars. A Log Rank (Mantel-Cox) showed no significant difference between the curves (p=0.168).

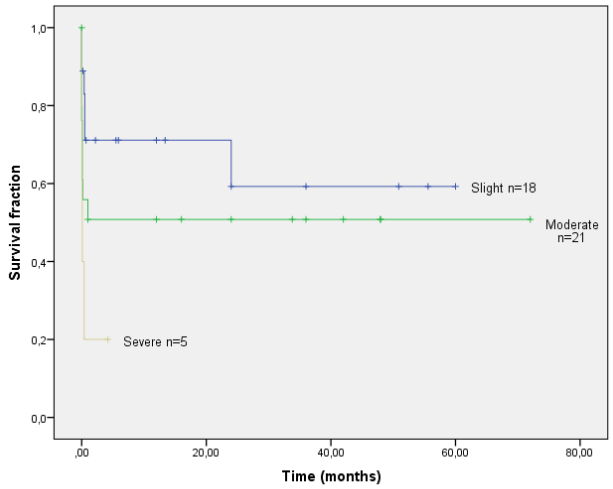

Figure 9 Kaplan-Meier curve for the survival time (months) cats diagnosed with FHL (n=44; 2003-2010. Cats were grouped based on cytological or histological examination indicating slight, moderate or severe lipidosis. Censored cases are indicated by vertical bars. A Log Rank (Mantel-Cox) showed no significant difference between the curves (p=0.109).

Prognostic factors for shortened survival time

Variables were screened for prognostic value by means of a univariate Cox's proportional-hazard analysis (table 9). Hypoalbuminemia appeared to be the only statistical significant (p<0,001) prognostic variable for shortened survival time with a hazard ratio of 9.356. Sex, severity of lipidosis, elevated bile acids and decreased β1 and β2 proteins approached significance (p<0.10).

Table 9 Univariate analysis for survival time after initial diagnosis n=44; 2003-2010)

Variable	No.	Events	P	HR	95% CI
Signalment					
Sex	44	20	0,087	0,657	0,406-1,062
Age	44	20	0,187	1,078	0,964 -1,205
Clinical signs					
Anorexia	44	20	0,66	1,219	0,505-2,944
Reduced appetite	44	20	0,499	1,362	0,556-3,336
Vomiting	44	20	0,636	1,241	0,507-3,038
Concurrent diseases	44	20	0,124	0.498	0.205-1.211
BCS 1	15	7	0,824	1,205	0,233-6,223
BCS 2	15	7	0,88	1,123	0,251-5,027
BCS 5	15	7	0,88	1,177	0,141-9,840
Lethargie	44	20	0,841	1,096	0,447-2,685
Consciousness (sopor)	44	20	0,231	1,31	0,842-2,038
Jaundice	44	14	0,286	0,499	1,39-1,790
Tachypnea	35	15	0,696	1,225	0,443-3,383
Tachycardia	38	16	0,222	0,516	0,179-1,493
Hypothermia	39	16	0,217	2,546	0,577-11,227
Hematology					
PT (Prolonged)	18	9	0,98	3,118	0,812-11,977
APTT (Prolonged)	18	9	0,981	0,984	0,262-3,694
Fibinogen (decreased)	18	9	0,24	2,594	0,530-12,705
Anemia	29	15	0,355	1,616	0,584-4,471
Lymphocyt count (low)	28	14	0,106	3,443	0,768-15,429
Segmented neutrophil (high)	28	14	0,606	1,709	0,223-13,081
Eosinophil count (low)	28	14	0,41	1,584	0,530-4,735
Clinical chemistry					
Uremia	28	12	0,563	1,404	0,444-4,433
Creatinin (high)	30	14	0,8	1,162	0,364-3,708
Bile acids (high)	32	13	0,09	5,852	0,759-45,147
ALT (high)	23	8	0,273	3,238	0,396-26,495
AP (high)	6	3	0,519	34,062	0,001-1535341
Sodium (low)	29	14	0,379	1,614	0,556-4,685
Potassium (low)	29	14	0,372	0,607	0,202-1,819
Calcium (low)	18	9	0,349	1,946	0,483-7,837
Phosphate (low)	19	9	0,606	1,442	0,359-5,785

Chlorine (low)	17	10	0,967	0,974	0,274-3,455
Magnesium	4	2	0,271	38,005	0,59-24653
Hypoproteinemia	33	16	0,116	2,282	0,816-6,380
Hypoalbuminemia	32	16	0	9,356	2,794-31,334
α2 (high)	21	9	0,864	0,871	0,181-4,206
β1 (low)	21	9	0,08	0,153	0,019-1,250
β2 (low)	21	9	0,091	3,189	0,832-12,217
γ (high)	21	9	0,421	1,909	0,396-9,208
Ultrasongrapic examination					
Enlarged liver size	44	20	0,949	1,029	0,428-2,476
Hyperechoic parenchym	44	13	0,378	1,142	0,850-1,535
Abnormal biliary system	44	20	0,674	0,768	0,224-2,627
Abdominal effusion	44	20	0,68	0,654	0,087-4,912
Pancreas abnormalities	44	20	0,449	1,528	0,510-4,582
Abnormalities in other organs	44	20	0,434	1,429	0,584-3,498
Cytological and histopathological examination					
Severity	44	20	0,064	1,846	0,966-3,530
Slight lipidosis	44	20	0.159	0.502	0.192-1311
Moderate lipidosis	44	20	0.656	1.221	0.507-2.941
Severe lipidosis	44	20	0,97	2,567	0,842-7,822

Discussion

Forty-four cats referred between 2003 and 2010 were included in this study by cytological or histopathological diagnosis of hepatic lipidosis and data was collected to characterize the population of cats suffering from hepatic lipidosis. The prevalence of FHL was higher than previously reported, 0,48% to 0,06%[14] and 0,16%[2]. The fact that our cases were part of a referral population in contrast to those of previous reports, in which primary care practices were used, probably explains this difference. However, the true prevalence of FHL might be underrated since not all cats undergo biopsy of the liver but slight or moderate hepatic lipidosis were reported to be common in ill cats[1]. Female cats are slightly more represented than male cats in this study. This could indicate that female cats are more prone to develop FHL than male cats as reported by previous results[1]. Middle aged cats were far more prevalent than young or old cats, 66 to 11 and 23 percent respectively. Age of cats ranged from 0,5 to 18 years old. These results are in line with previous reported findings[1]. The FHL cats in this study were of 11 different breeds of which the European shorthair comprised the majority (64%) of cats, whereas the other breeds contained 11% or less cats. Additional analysis of the total population of cats referred to the clinic from 2003 to 2010 should give more insights in these results.

The most common complaints (>50%) reported by the owner on initial questionnaire were lethargy, a history of weight loss and anorexia. Weight loss ranged from 3 to 48% of bodyweight in 27 cats with a median of 21%. An average weight loss of 25% of bodyweight is previously mentioned in cats with severe FHL[1]. It would seem that the percentage of weight loss does not correlate with severity of FHL since only 5 (11%) cats in this study were classified as having severe lipidosis. A more in-depth analysis of the amount of weight loss per time unit for each cat could give a better indication of the importance of this development. Few cats were scored as obese with the majority (80%) considered to be underweight or very thin. This finding is in contrast with that of previous reports of obesity being a predisposing risk factor for FHL[1,2,9]. However, a scoring of the body condition has not always been part of the physical examination routine and therefore overweight or obese cats might have been missed.

Less frequent complaints (<50%) were reduced appetite, vomiting, neurological signs and jaundice. The median and range of days of anorexia prior to initial examination was remarkably lower than that of reduced appetite. Considering the impact of anorexia compared with reduced appetite these findings should not be surprising. However reduced appetite was also reported prior to anorexia in some cases (excluded from results). This may well have contributed to the extent of clinical signs when full anorexia did occur. In any case, not only anorexia, but reduced appetite itself is also an important factor in the development of FHL[1]. Longer periods of reduced appetite in the cat should therefore be an alarming clinical sign for owner as well as veterinarian.

Vomiting was a common sign (>50%) in previous reports of FHL[1,2] but is only reported in 34% of cases in this study. Other gastrointestinal signs such as diarrhea or constipation are also not prevalent in this study compared to the same reports[1,2]. This difference might be explained by the large fractions of cats with slight and moderate lipidosis, since obvious signs of FHL become more predominant in severe lipidosis. Neurological signs were uncommon, only once behavioral change was observed and one cat had a history of headtremors. Concurrent diseases were reported in a large fraction of cats. These consisted mostly of cholangitis, pancreatitis, neoplasia and diabetes mellitus and are reported to be related to FHL[2,9]. Further analysis of diagnosed concurrent diseases during clinical workup is

advised to give an indication of cats with primary or idiopathic FHL and secondary FHL. However, it must be noted that some cats died before a complete clinical workup could be performed. In all, differentiation between these two types of FHL remains a precarious task.

Clinical examination showed that most cats were tachypnoeic, tachycardic and had an bodytemperature slightly lower than the reference interval. Jaundice was seen in 34% of cats. This percentage is considerably higher than reported in the clinical history (14%). A reason for this maybe that owners are not always perceptive of this clinical sign. Also, jaundice was usually reported as icteric membranes and might not have been severe enough (i.e. color change of skin and white of eye) for the owner to notice.

Coagulation profile was abnormal in at least one variable in 50% of cats with FHL. Nine cats had a complete coagulation profile (i.e. PT, APTT, Fibrinogen and Platelet count) of which only two cats showed abnormal values in PT, APTT and fibrinogen. However, none of the cats reported signs of bleeding tendencies (i.e. bruising, extensive bleeding after venipuncture). This discrepancy between laboratory results and clinical findings has been reported before[1]. A possible explanation is that preventative vitamin K administration might have prevented bleeding from occurring. However, only three cats in this study have been reported with administration of vitamin K. Noticed abnormalities in the coagulation profile did not differ much from the reference interval, except for a prolonged APTT. This can partially be explained by the use of a general reference interval for this variable instead of a local established reference interval determined by the clinic as is done for most diagnostic tests. The investigated packed cell volume was decreased in 45% of cats which is common in chronic diseases such as FHL[1]. In contrary to previous results, a decrease in PCV with similar median and range was not associated with shortened survival time (p=0,355). In most cats (>80%) a normal WBC count was measured with decreased lymphocyte and eosinophil counts and slightly elevated segmented neutrophils and might be due to stress caused by disease or handling by owner and/or veterinary staff (stress leukogram)[15].

Bile acids, ALAT , GGT and AP (activity) were elevated in most cats (>50%) with FHL. This is consistent with liver disease in general and with FHL[2]. When comparing values between the sexes, female cats appeared to have a higher incidence in elevated bile acids, ALAT activity and AP. Relative higher levels of bile acids could suggest that female cats are prone to develop more severe liver disease than male cats. Gastrointestinal signs in FHL cats reported here, in combination with decreased intake (reduced appetite/anorexia) are common causes of decreased levels of sodium and potassium[16]. The decrease in calcium is unusual for FHL but can indicate an underlying disease such as pancreatitis[17]. The increase of chloride in cats with FHL can be caused by an excess loss of sodium (due to diarrhea) or excessive gain of chloride (KCL treatment). Moreover, renal retention of chloride with diabetes mellitus or renal failure can be a cause. Previous results showed similar electrolyte values, although sodium, potassium and calcium values were distributed around the lower reference interval limit instead of having a wider range covering both reference limits as reported by Center et al[1].

Total protein concentration in FHL cats was generally within reference limits, although albumin concentration was considerable lower. The elevated α2 en γ globulins compensating for the decrease in albumin may create a seemingly normal total protein value. This underscores the importance of analysis of total protein as well as albumin. The majority of FHL cats had an hypoalbuminemia or had

albumin levels in the lower range of the reference interval. Low albumin levels are often seen in hepatic disease and can indicate a lower production either by impaired liver functions or an decreased protein uptake[17]. Also an hyperglobulinemia can cause hypoalbuminemia due to down regulation. The increase in globulins (polyclonal) can be explained by concurrent inflammatory diseases such as cholangitis. In addition infectious diseases such as FIV, FeLV and FIP induce in increase in globulins. However, in this study no cat tested positive for FIV and FeLV and no cat was diagnosed with FIP. Two cats had high levels of thyroxine (T4) and were diagnosed with hyperthyroidism. Elevated fPLI was measured in 4 out of 5 cats indicating pancreatitis. However, this measurement became available during the period of investigation so the true prevalence may be underrated.

More than 50% of the FHL cats showed an enlarged liver and hyperechoic parenchyma on ultrasonografic examination. Hepatomegaly is commonly seen in chronic cases of hepatic lipidosis but may not be apparent in acute cases[2]. Hyperechoic changes however are indicative of hepatic lipidosis although other hepatic diseases can also cause these changes[18]. Furthermore, normal obese cats can also show a hyperechoic liverparenchyma[19]. Seven cats showed abnormalities in the biliary sytem of which an number of cats were diagnosed with concurrent cholangitis or cholangiohepatits. Abnormalities in the pancreas were seen in 7 cats, giving the suspicion of pancreatitis in these cats.

The inclusion criteria for this study was the diagnosis of hepatic lipidosis, which was performed by cytologic or histopathological examination. One cat showed no lipidosis on cytological examination but had slight lipidosis on the histopathoglogical examination of a biopsy 24 hours later. Also one cat reported moderate lipidosis on cytological examination and slight lipidosis on histopathological examination. This may indicate that histopathological examination is more sensitive. However, cytological examination is reliable in the diagnosis of hepatic lipidosis and other vacuolar diseases[20]. Furthermore, one cat with also no lipidosis reported on a first cytological examination was diagnosed with slight lipidosis 24 hours later on a subsequent cytological examination, which might indicate that lipidosis can occur within 24 hours in some cases. Despite these discrepancies in results between cytology and histopathology, 3 of the 5 cats with both methods had similar results. Concurrent processes were seen in seven cats, none of them examined by both methods. Four reported signs of inflammation, 2 cats showed neoplastic cells and one eastern shorthair cat was diagnosed with amyloidosis. Focal lesions are not always detected by cytology and concurrent diseases may be overseen.

Treatment was not evaluated in the study because each cat received a different therapy according to their concurrent disease (e.g. cholangitis, pancreatitis). Some were treated at home by the owners while others were admitted to the intensive care for additional treatment such as fluid therapy. In all cases forced feeding was implemented or advised.

Clinical outcome was determined by clinical reports or by follow up. Seventeen cats showed remission of clinical signs with 4 cats having recurrent episodes of clinical signs. Clinical signs could reoccur within a short period (e.g. one week), although one cat showed recurrence after two years. Recognition of the clinical signs by the owner and direct administration of treatment seemed to reduce the period of clinical signs from 3 months (at initial diagnosis) to one week. Twenty-three cats had persistent signs until death of which 19 were FHL related. Three cats were alive at the time of follow up and 37 cats had died with 4 cats lost to follow up. Twenty cats were determined having a

FHL related death, i.e. having mainly clinical signs fitting the profile of FHL prior to death, independent of concurrent disease. Unfortunately some discrepancy can occur between clinical signs fitting FHL and a definitely diagnosis of FHL. One cat had a recurrent episode with signs of FHL, was euthanized shortly after because of these signs and was referred for necropsy. The histology report following the necropsy showed no lipidosis but another vacuolar hepatic disease. In order to increase the reliability of these results cats with presumable lipidosis related deaths should be referred for necropsy. Costs for the procedure and emotional reluctance of the owner often withheld further absolute diagnosis in this study.

Survival time was calculated as the period in months from initial diagnosis till death. FHL unrelated deaths, cats lost to follow up and cats that were still alive were considered censored cases. Unfortunately more than half of the cats were censored (n=24) so no estimated median survival time (EMST) could be calculated for the overall FHL population. An EMST could only be calculated for severe lipidosis and is 0.1 month with a confidence interval of 0-0.207. Almost all cats (n=19) died within the first month after initial diagnosis of which 14 died within one week . This high mortality rate is probably due to the fact that these cats already have a history of clinical signs (and were already deteriorating) and period of disease before referral to the clinic. Furthermore, some owners decided to directly euthanize the animal due to the inability to maintain treatment for several weeks or months and the already extensive period of disease with a uncertain prognosis of recovery. Although no significant difference was present for shortened survival time, female cats seem to have more risk for an early death (p=0.064). However, more male cats were censored (13 of 19 cats) than female cats (11 of 25 cats) and had consequently fewer numbers (6 males to 14 female) to be taken into the graph. The lower survival rate in females may coincide with the increased concentrations of bile acids. Follow up studies with larger number of cats with FHL could give a better depiction. No significant difference in survival times between young (0-5 years), middleaged (6-12) and senior (>12 years) FHL cats was present. However all three cats that were still alive at follow up were 5 years or younger at initial diagnosis supporting the conclusion from earlier studies that younger cats with FHL have an higher survival rate[1]. The different severities of lipidosis also did not have a significant difference in survival time. As mentioned earlier most cats died within one month of initial diagnosis. Only one cat died after the first month and was diagnosed with slight lipidosis. Hypoalbuminemia was found to be a prognostic factor for shortened survival time (p<0,001) in cats with FHL with a hazard risk of 9.356. Eleven of 16 cats with FHL related deaths had hypoalbuminemia of which most were female (n=9). Earlier studies did not come to this conclusion. A possible explanation for this discrepancy can be that the low level of albumin reflects the severity of the disease and clinical signs that can influence the owners decision for treatment or euthanasia. Also the number of cats is considerable smaller and less uniform than that of in the earlier study of FHL by Center et al[1].

The retrospective design of this study was self-limiting. However, we were able to detect several parameters that describe our population of FHL cats. In summary, lethargy, weight loss and anorexia are common complaints with gastrointestinal signs occurring in less than 50%. Females appear to be more affected than males and body condition seems to be less than ideal in contrast to overweight or obesity reported earlier. Blood examination revealed that anemia in FHL cats is common and that levels of bile acids, ALAT, GGT and AP are frequently increased with electrolyte concentrations predominantly decreased. Hypoalbuminemia and slight hyperglobulinemia is prevalent and a decreased albumin is a significant prognostic factor for decreased survival time (p<0,001). PCV and

hypokalemia were not significant as prognostic factors contradicting earlier results in FHL cats[1]. Sex, age and severity of lipidosis do not significantly influence survival rates.

Well known is that FHL is a syndrome that is often secondary to a underlying concurrent (hepatic) disease. The influence of concurrent diseases on the results described is difficult if not impossible to distinguish. A more in depth analysis of primary and secondary hepatic lipidosis might solve this problem. Furthermore, the apparent idiosyncrasy seen in females compared to males with FHL could be clarified with additional research comprising a larger number of cats.

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