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Primary Hepatitis in Dogs: A Retrospective Review (2002–2006)

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Background: Little is known about etiology, disease progression, treatment outcome, survival time, and factors affecting prognosis in dogs with primary hepatitis (PH).

Objectives: To review retrospectively different forms of hepatitis in a referral population, by the World Small Animal Veterinary Association Standardization criteria.

Animals: One-hundred and one dogs examined for histologically confirmed PH between 2002 and 2006. Dogs with nonspecific reactive hepatitis were excluded.

Methods: Retrospective study. Medical records were reviewed for prevalence, signalment, clinical and clinicopathologic manifestation, outcome, survival time, and prognostic factors for shortened survival.

Results: PH occurred in 0.5% of dogs in this referral population. Acute (AH) and chronic hepatitis (CH) were diagnosed in 21 and 67 dogs, respectively. Progression from AH to CH occurred in 5/12 of the repeatedly sampled dogs. CH was idiopathic in 43 (64%) dogs, and was associated with copper accumulation in 24 (36%) dogs. Median survival time was longer in dogs with AH than in dogs with CH (either idiopathic or copper associated), and dogs with lobular dissecting hepatitis had the shortest survival time. Prognostic factors predicting shortened survival were associated with decompensated liver function and cirrhosis at initial examination.

Conclusions and Clinical Importance: The majority of PH in dogs is CH. Previous studies appear to have underestimated the etiologic role of copper in both AH and CH. Prognosis is reduced in dogs with hepatic cirrhosis or cirrhosis-related clinical findings. Further research into etiology and treatment effectiveness in all PH forms is needed.

Key words: Copper-accumulation; Dogs; Primary hepatitis; Prognostic factors; WSAVA histopathology criteria.

Primary hepatitis (PH) is the most frequently occurring group of liver diseases in dogs, and comprises all inflammatory hepatic diseases that are not characterized by nonspecific changes, as observed in nonspecific reactive hepatitis.[1] In contrast to humans, the diagnosis of hepatitis in dogs is mainly based on histologic morphology, and the term is often used regardless of the etiology. Regularly encountered forms of PH in dogs include acute (AH) and chronic hepatitis (CH, with or without cirrhosis); less frequently encountered are lobular dissecting (LDH), granulomatous (GH), and eosinophilic hepatitis (EH). The World Small Animal Veterinary Association (WSAVA) Liver Standardization Group has published standards for the diagnosis for each of these forms.[1]

A range of causes of hepatopathy have been documented in different publications and case reports, including microorganisms,[2–14] toxins and drugs,[15–22] immune-mediated reactions,[23–26] and breed-associated metabolic errors.[27–29] The inherited disorders of copper metabolism received particular attention in the last few decades.[30–35] In spite of significant research efforts, the causes remain elusive.[36] No published data are available on (1) the occurrence of the various forms of the disease in a clinical population, (2) its progression from acute to chronic forms, (3) the occurrence of idiopathic and copper-associated forms of hepatitis, nor (4) on survival and prognostic factors for all forms of PH, including AH, CH, and LDH.[37–39]

The purpose of this study was 4-fold. Firstly, to describe different forms of hepatitis in our clinical referral population, including clinical, laboratory, and pathologic findings. Secondly, to assess the frequency of copper accumulation as a potential cause in all these forms of hepatitis. Thirdly, to evaluate progression of AH to CH, and of CH to cirrhosis. And, fourthly, to assess the clinical outcome and survival time after diagnosis, and prognostic factors for shortened survival time.

Material and Methods

Study Design

Retrospective review of medical records.

Dogs

All dogs in this study were referred between 2002 and 2006 to the Department of Clinical Sciences of Companion Animals, University of Utrecht, the Netherlands. The dogs were identified from the records of the diagnostic pathology service at the Faculty of Veterinary Medicine. Included were dogs with a form of histology-proven PH as described by the WSAVA liver standardization group.[1] Excluded were dogs if they had secondary hepatic inflammation, such as nonspecific reactive hepatitis or ischemic injury with secondary inflammatory reactions, based on histopathology of liver biopsy samples. Data concerning signalment, medical history, clinical, laboratory and histopathologic findings were obtained from the medical records. Histopathology was reviewed as described below. All procedures were approved by the University's ethical committee as required under Dutch legislation.

Histopathology

At the time of original diagnosis, at least 2 liver biopsy samples per occasion were obtained from each dog by percutaneous biopsy with a 14 G needle (blind Menghini biopsy or true cut biopsy under ultrasonographic guidance).[40] Histology of all identified cases was reviewed by 1 board-certified veterinary pathologist according to

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the WSAVA-criteria.[1] Slices of formalin-fixed, paraffin-embedded specimen were stained with hematoxylin and eosin (HE), reticulin according to Gordon and Sweet, and rubeanic acid. Several variables were evaluated semiquantitatively, including necro-inflammatory activity and presence of apoptosis (0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe), fibrosis (0 = none, 1 = focal, 2 = bridging, 3 = bridging with architectural distortion or cirrhosis), and copper content and distribution, by a previously reported copper grading system.[41,42] Copper scores >2 are considered abnormal and a potential etiologic factor.[43] Presence of steroid-induced hepatopathy (SIH) was also evaluated. Consequently, dogs were categorized into AH, CH, LDH, GH, or EH. A group of miscellaneous hepatic disorders was included, which had not been diagnosed with hepatitis initially, but progressed to CH later, as proven by repeated histopathologic exams. All dogs in the AH and CH groups were judged as either idiopathic (i) or copper associated (ca) according to the semiquantitative evaluation for copper (copper $\leq$ or $>$ 2, respectively). No quantitative copper analysis was performed. Postmortem examination was rarely performed; thus those results are not reported.

Follow-Up

Data were collected during February 2007 by telephonic interviews with the owners or referring veterinarians, regarding clinical progress with or without continuation of medication. Data included clinical outcome, including residual disease, remission or recurrence of hepatitis-related clinical signs, and death, survival time after diagnosis, and the presumed cause of death, where applicable.

Evaluated Variables

The variables included for description and statistical analysis are presented in Table 1. Not all blood variables are described for the study sample, because some values for some variables were missing.

Table 1. Evaluated variables that were collected from medical records and histopathology reviews (n = 101, 2002–2006).

Data Categories	Collected Variables
Signalment and history	Age, sex, clinical signs and duration, medical history
Biochemistry markers	Total alkaline phosphatase (ALP), steroid-induced alkaline phosphatase (ALP-65), alanine aminotransferase (ALT), fasting total serum bile acids (sBA), urea, creatinin, glucose, total serum protein (TP), albumin, serum ammonia (NH_3), sodium, potassium, calcium, anorganic phosphate, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen
Hematologic markers	Hematocrit (Ht), leukocytes and differentiation, thrombocytes
Ultrasonographic findings	Hepatic size, surface regularity, and structure regularity and echodensity; presence of portosystemic collateral shunts and ascites, gallbladder changes, and portal lymph node size
Histopathologic findings	Semiquantitation of necro-inflammatory activity, fibrosis and cirrhosis, grading for copper content and distribution; and presence of steroid-induced hepatopathy
Treatment	Medical therapy; type and duration
Follow-up	Outcome after treatment (complete remission or recurrence of clinical signs, or residual disease), date of death, and presumable cause of death (clinical signs before death related to hepatitis or not)

Statistical Analysis

Analysis was performed by a commercially available software package.[a] The χ^2 test was performed for goodness-of-fit of the study group compared with the total canine clinical population (2002–2006) for breed and sex distribution. A 1-sample Kolmogorov-Smirnov test was used to assess normality of all data. A nonparametric Kruskal-Wallis *H*-test was performed for comparison of ordinal mean values in case of 3 groups or more. A 1-way ANOVA test with a posthoc Bonferroni's correction was performed for comparison of continuous values (age and blood measurements) in case of 3 groups or more, and a Levine's test was used for determining the homogeneity of variances. The log-values of hematologic and serum biochemical values were used owing to nonnormal distributions and frequent outliers. Survival fractions were calculated according to the Kaplan-Meier procedure. The survival time was defined as the interval between the date of initial diagnosis and the date of death. Dogs that died of hepatitis-related causes were counted as events. Dogs that died of unrelated causes or that were alive at follow-up were censored. Differences between Kaplan-Meier curves were tested for significance by a log-rank test. Prognostic factors for shortened survival time were identified by combined analysis of survival time and variables retrieved from the medical records and histopathologic reviews by Cox regression models. These variables were screened with a univariate Cox's proportional-hazard analysis to assess which variables were suitable for further analysis. All variables that were significant or approached significance ($P < .10$) were entered into a stepwise multivariate Cox's proportional-hazard analysis with backward removal of variables (likelihood ratio). Various multivariate analyses were executed, because not all analyzed variables were available for all dogs included in the study. Those with lower numbers of cases and events were each entered separately for maximal output, and to prevent an unnecessary reduction of cases.[44] Owing to small group size, variable analysis was performed for the entire study population, and not for each form of hepatitis. Descriptive and group comparative data are presented as mean $\pm$ SD (range) when normally distributed, and median (range) when not normally distributed; survival data as estimated median survival times (EMST) $\pm$ SD (95% confidence interval, 95%CI); and Cox regression analysis as hazard rates (HR), and 95% CI. All statistical tests were considered significant at the 5% level ($P < .05$), unless stated otherwise.

Results

Prevalence and Progression

One hundred and one dogs with PH were identified, which represented 0.5% of the entire number of 1st visits of our clinic population in the same period. Most frequently observed was CH (67/101), consisting of 43/67 dogs with idiopathic CH (CH[i]) and 24/67 dogs with copper-associated CH (CH[ca]) (Table 2). Hepatic cirrhosis was found in all chronic groups; CH(i) (24/43), CH(ca) (13/24), and LDH (7/7). Four dogs did not have an initial diagnosis of PH, but developed PH at a later stage. Therefore, these cases are marked "miscellaneous." Excessive hepatic copper was found in 29/88 dogs with AH (5/21) and CH (24/67).

Forty-eight dogs returned to the university clinic for a repeated liver biopsy approximately 6 weeks after the initial biopsy. Of the 21 AH dogs, 12 returned for a 2nd biopsy; histopathology revealed complete remission of inflammatory and degenerative changes in 6 dogs, no changes in 1 dog, and progression from AH to CH in 5 dogs. Of the 37 initially cirrhosis-free CH dogs, 2

Table 2. Dog breeds and frequency of primary hepatitis of differing form.

Dog Breeds	AH (i)	AH (ca)	CH (i)	CH (ca)	LDH	GH	EH	Miscellaneous
Airdale Terrier		1						
American Cocker Spaniel			2	1				
Bedlington Terrier		1		1				
Bernese Mountain Dog			1		1			
Border Terrier			1				1	
Boxer	1		1					
Cairn Terrier				2				
Cavalier King Charles Spaniel			1	1				
Dobermann Pincher	1			1				
German Pointer			3[a]					
English Cocker Spaniel			2	2	1			1
Fox Terrier	1				1			
Golden Retriever			3	1		1		
Jack Russell Terrier	3				1			
Crossbreeds	2		5	3				
Labrador Retriever	2		11	5				1
Scottish Terrier			2					
Stabyhound			1		1			
West Highland White Terrier	1		2	2				
Incidental cases in other breeds (n = 25)	5	3	8	5	2	0	0	2
Total number of dogs	16	5	43	24	7	1	1	4

[a]In 1 dog *Leishmania*-associated lesions were found.

AH, acute hepatitis; CH, chronic hepatitis; LDH, lobular dissecting hepatitis; GH, granulomatous hepatitis; EH, eosinophilic hepatitis; i, idiopathic; ca, copper associated.

revealed progression to cirrhosis upon examination of follow-up biopsies. All 4 dogs diagnosed with miscellaneous hepatitis progressed to CH; 1 of them also developed cirrhosis.

Etiology

Of the 21 AH and 67 CH dogs, 5 and 24—respectively—had copper scores higher than 2 (reference ≤ 2), and were considered copper associated. *Leishmania*-induced hepatic lesions were found in the repeated liver biopsy sample of 1 CH dog, which was therefore diagnosed with *Leishmania*-associated CH. The remaining 16 AH and 42 CH dogs were considered idiopathic. Eight dogs, mainly in the CH group, were treated with potentially hepatotoxic drugs before referral or for treatment of concurrent disease, including short-term administration of antibiotics (TMP/S, n = 1) and NSAIDs (n = 5), and chronic administration of phenobarbital (n = 2); no idiosyncratic hepatic changes were found upon histopathologic examination. None of the other dogs have been treated with potentially hepatotoxic drugs in the months before presentation. Leptospirosis was suspected in 5 dogs, but only 1 dog had high IgG titers (1 : 160) and normal IgM titers (1 : 20) upon serologic examination. In the LDH and GH groups no potential etiologies were found with histology or clinical pathology. In the single dog with EH, histology revealed parasitic migrating tracks in close proximity to eosinophilic changes.

Breed, Sex, and Age

Forty-three purebred (n = 91) and crossbred (n = 10) breeds were encountered (Table 2). The breed distribution of the study population was significantly ($P < .001$) different from the total clinic population. Overrepresented were English and American Cocker Spaniels, Labrador and Golden Retrievers, West Highland White and Jack Russell Terriers, and German Pointers. The sex distribution in the entire study population was significantly ($.001 < P < .01$) different from the clinic population, with an overrepresentation of females (25 intact and 43 neutered females; 22 intact and 11 castrated males). Individual forms of PH indicated a sex difference in the AH(i) group ($P < .001$), with an overrepresentation of neutered females (n = 11), and in the LDH group ($P < .025$), with an overrepresentation of intact females (n = 5). Age (years, mean $\pm$ SD) was distributed as follows: AH(i) 7.8 $\pm$ 4.1 (range, 2.4–14.5), AH(ca) 5.9 $\pm$ 3.2 (range, 1.4–9.0), CH(i) 7.7 $\pm$ 3.4 (range, 0.4–14.2), and CH(ca) 6.5 $\pm$ 3.4 (range, 0.9–13.8). Mean age at presentation was lower for LDH (2.3 $\pm$ 2.5, range, 0.5–7.2), GH (3.4), and EH (0.6). The LDH group had a significantly lower mean age in comparison with the AH and CH groups ($P < .05$).

Clinical Signs and Medical History

Clinical signs and physical examination findings in the 101 dogs were lethargy (56/101), anorexia (56/101), vomiting (48/101), polyuria and polydipsia (47/101), weight loss (28/101), coffee-colored urine (27/101), jaundice (24/101), diarrhea (23/101), abdominal distention (21/101), signs of hepato-encephalopathy (HE, 22/101), hepatomegaly (17/101), urinary incontinence (15/101), abdominal fluid wave (9/101), pruritis (9/101), and abdominal pain (8/101). Signs related to portal hypertension (HE and ascites) occurred only in dogs with histology-proven cirrhosis. Fifty-four of all dogs had increased liver enzymes and/or bile acid concentrations before referral, and chronically increased liver enzymes were the only reason for referral in 2 dogs that were free of clinical signs. Clinical signs were present during 10.0 $\pm$ 15.0 weeks (range, 0.5–52) for AH, 9.6 $\pm$ 18.9 weeks (range, 0.5–104) for CH(i), 4.3 $\pm$ 3.8 weeks (range, 0.5–14.0) for CH(ca), and 5.1 $\pm$ 6.3 weeks (range, 1–19) for LDH. No significant difference was found between the groups for the duration of clinical signs.

Twenty-five dogs had a history of concurrent disease and were treated accordingly. They presented with epilepsy (4/25), diabetes mellitus (2/25), pituitary-dependent hyperadrenocorticism (3/25), hypothyroidism (1/25), hyperthyroidism (1/25), arthritis/arthrosis (2/25), chronic kidney insufficiency (1/25), signs of chronic large or small bowel diarrhea (4/25), keratoconjunctivitis sicca (2/25), signs of unidentified heart disease (2/25), atopic dermatitis (2/25), and pseudopregnancy (1/25).

Hematologic and Serum Biochemistry Findings

Several 1st consultation biochemical and hematologic markers measured are summarized in Table 3. The markers not presented did not differ significantly between the analyzed groups. Bilirubin levels were rarely measured in the study population, and were therefore not included. Blood coagulation was abnormal in 18 dogs, resulting in postponement of their liver biopsy procedure. All of these dogs were treated with prednisone during 1 week, which normalized coagulation variables, permitting harvesting of biopsies. Values of all variables were statistically compared between the groups. The LDH group had a lower mean total serum protein value than the other 3 groups ($P < .01$). The CH(i) group had a lower glucose concentration than dogs with CH(ca) ($P = .016$). No significant differences were found upon comparison of the mean values above or below the reference range of each variable.

Ultrasonographic Examination Findings

Of the 97 dogs that had ultrasonographic examinations, 2/21 AH, 15/67 CH, and 2/7 LDH had no abnormalities. Hepatic size was assessed as normal (AH—7/21; CH—26/67), enlarged (AH—8/21; CH—15/67), or too small (AH—6/21; CH—26/67). Hepatic surface was mostly assessed as normal (AH—19/21; CH—54/67). Hepatic structure was found to be either normal (AH—7/21; CH—27/67), irregular (AH—11/21; CH—31/67), or with increased echodensity (AH—3/21; CH—11/67). Nodular processes were observed mostly in chronic forms of PH (AH—2/21; CH—16/67; LDH—1/7; GH—1/1). Ascites were also predominantly found in chronic groups (AH—3/21; CH—18/67; LDH—4/7), as well as portosystemic collaterals (AH—6/67; LDH—1/7). Enlarged portal lymph nodes were infrequently observed (AH—1/21; CH—7/67; GH—1/1), as well as abnormal gallbladder walls (AH—7/21; CH—7/67; LDH—1/7).

Histopathologic Findings

The histological semiquantitative scoring of liver samples at initial diagnosis is summarized in Table 4. Necro-inflammatory activity was not significantly different ($P = .27$) between the groups. Fibrosis extent was significantly higher ($P = .020$) in the LDH group compared with CH(i) and CH(ca). Copper was not

Table 3. Liver-related and other selected serum biochemical and hematologic markers at initial diagnosis (n = 95; 2002–2006).

	Serum Biochemical Markers							
Variable	sBA	ALP	ALP-65	ALT	Glucose	TP	Albumin	NH_3
RR	< 10	< 73	< 15% AP	16–69	4.5–5.8	53–66	28–36	24–45
Unit	μmol/L	U/L	U/L	U/L	mmol/L	g/L	g/L	μmol/L
AH (total)	13/18 (↑)	12/14 (↑)	13/13 (↑)	8/8 (↑)	2/6 (↓)	3/12 (↓)	5/12 (↓)	0/5 (-)
Mean ± SD	108.0 ± 190.0	982.5 ± 962.6	335.3 ± 691.0	1055.5 ± 1539.5	5.8 ± 2.4	57.1 ± 10.1	24.9 ± 5.9	15.8 ± 8.3
CH(i)	32/35 (↑)	32/33 (↑)	26/26 (↑)	16/17 (↑)	2/8 (↓)	5/23 (↓)	15/24 (↓)	1/7 (↑)
Mean ± SD	75.7 ± 79.6	660.3 ± 728.8	238.7 ± 450.0	403.4 ± 340.6	4.9 ± 1.4*	57.9 ± 7.9	26.0 ± 4.5	33.9 ± 21.3
CH(ca)	13/19 (↑)	15/16 (↑)	10/10 (↑)	5/5 (↑)	0/5 (-)	5/13 (↓)	7/14 (↓)	3/8 (↑)
Mean ± SD	45.6 ± 61.7	496.1 ± 605.0	255.9 ± 286.8	430.0 ± 221.6	10.4 ± 5.1*	54.8 ± 10.8	26.9 ± 6.2	47.0 ± 47.1
LDH	6/6 (↑)	5/6 (↑)	5/5 (↑)	2/3 (↑)	0/0 (-)	3/3 (↓)	3/4 (↓)	3/4 (↑)
Mean ± SD	49.8 ± 42.3	319.8 ± 156.2	10.2 ± 4.1	134.0 ± 94.0	–	37.7 ± 7.4**	20.3 ± 8.1	56.5 ± 33.5

	Hematologic Markers					Coagulation Markers		
Variable	Ht	Leukocytes	Monocytes	Segments	Thrombocytes	PT	APTT	Fibrinogen
RR	0.42–0.61	4.5–14.6	0–0.9	2.9–11	144–603	6.7–9.5	10–17.2	1–2.8
Unit	L/L	$x10^9$/L	$x10^9$/L	$x10^9$/L	$x10^9$/L	s	s	g/L
AH (total)	5/11 (↓)	5/11 (↑)	4/11 (↑)	6/11 (↑)	2/5 (↓)	4/18 (↑)	5/18 (↑)	0/18 (↓)
Mean ± SD	0.42 ± 0.09	15.9 ± 8.8	0.9 ± 0.5	12.0 ± 7.9	164.4 ± 171.0	14.9 ± 28.8	18.7 ± 12.5	2.8 ± 1.9
CH(i)	11/21 (↓)	11/24 (↑)	8/23 (↑)	10/24 (↑)	4/10 (↓)	14/34 (↑)	12/34 (↑)	8/34 (↓)
Mean ± SD	0.40 ± 0.10	14.8 ± 10.2	0.9 ± 0.7	11.3 ± 9.5	201.7 ± 105.3	10.1 ± 3.9	17.1 ± 7.3	2.5 ± 2.9
CH(ca)	5/12 (↓)	5/12 (↑)	5/12 (↑)	5/12 (↑)	1/10 (↓)	6/20 (↑)	8/20 (↑)	7/20 (↓)
Mean ± SD	0.44 ± 0.09	20.2 ± 14.5	1.2 ± 1.0	16.6 ± 13.6	219.9 ± 68.9	9.9 ± 5.9	18.7 ± 6.4	1.8 ± 1.3
LDH	1/3 (↓)	1/2 (↑)	0/2 (-)	1/2 (↑)	0/2 (-)	5/6 (↑)	5/6 (↑)	1/6 (↓)
Mean ± SD	0.41 ± 0.04	15.2 ± 9.2	0.5 ± 0.4	13.1 ± 9.3	237.0 ± 29.7	11.2 ± 2.7	22.7 ± 4.9	1.4 ± 1.0

*Mean glucose value significantly lower ($P = .016$) for CH(i) when compared with CH(ca);

**Mean serum total protein value significantly lower ($P < 0.01$) for LDH when compared with the other groups.

RR, reference range; (↑), above reference range; (↓), below reference range; (−), within reference range; AH, acute hepatitis; CH, chronic hepatitis; i, idiopathic; ca, copper associated; LDH, lobular dissecting hepatitis; ALP, alkaline phosphatase; ALP-65, steroid-induced alkaline phosphatase; ALT, alanine aminotransferase; sBA, serum bile acids; TP, total serum protein; NH_3, serum ammonia; Ht, hematocrit; PT, prothrombin time; APTT, activated partial thromboplastin time.

Table 4. Histologic semiquantitative scoring of liver samples at initial diagnosis (n = 95; 2002–2006).

Diagnosis	AH(i)	AH(ca)	CH(i)	CH(ca)	LDH
No. of dogs	16	5	43	24	7
Inflammation					
Mean	2.2	2.3	2.0	2.5	2.1
(range)	(0–5)	(2–3)	(0–4)	(2–4)	(2–3)
Fibrosis (*extent*)					
Mean	0.0	0.0	2.2	2.3	3.0*
(range)	(0–0)	(0–0)	(0–3)	(1–3)	(3–3)
Copper score					
Mean	0.5	3.8	0.7	3.2	1.8
(range)	(0–2)	(3–5)	(0–2)	(2.5–4.5)	(0–3)

*Significantly higher ($P = .020$) semiquantitative fibrosis scores for LDH when compared with the other groups. Please note that 6 dogs were excluded owing to small group sizes.

No. = number; AH, acute hepatitis; CH, chronic hepatitis; i, idiopathic; ca, copper associated; LDH, lobular dissecting hepatitis.

significantly different ($P = .27$) between AH(ca) and CH(ca). Copper was distributed mainly in zone 1 (periportal) of the liver acini in the CH(i) group, and mainly in zone 3 (centrolobular) in the CH(ca) group. Cirrhosis was observed in the CH(i), CH(ca), and LDH groups (n = 23, 14, and 7, respectively), including macronodular cirrhosis (n = 7) in the CH(i) group and micronodular (n = 2) in the CH(ca) group. SIH was observed in 39 liver samples, mainly in the dogs with CH that had been treated with glucocorticoids.

Treatment and Clinical Outcome

All dogs were treated symptomatically and palliatively as indicated by their clinical status and disease. Treatment adjustments were made if histology of repeated biopsies resulted in a different diagnosis. Dogs with AH(i) were treated with antibiotics (5/16), hepatic-support diet and ursodeoxycholic acid (7/16), and 5/16 dogs were not treated. Dogs with AH(ca) were treated with prednisone (3/5) or D-penicillamine (2/5), supplemented by hepatic-support diet and oral zinc gluconate. Dogs with CH(i) were mainly treated with prednisone (29/43), a minority received only antibiotics or combined with prednisone (6/43), and 12/43 dogs were not treated owing to mild inflammatory activity. Dogs with CH(ca) were initially treated with prednisone (9/23), D-penicillamine (5/23), or not treated at all in case of mild activity (9/23). After a repeated biopsy, more dogs received D-penicillamine (10/23), oral zinc gluconate (2/23), and hepatic-support diet (5/23). Most LDH dogs were only treated symptomatically, 1 additionally with antibiotics, and 3 with prednisone. Lactulose, diuretics, and low-protein diets were prescribed in case of clinical signs of portal hypertension in all relevant groups. The single GH and EH dogs were not treated, because the former died shortly after diagnosis, and the latter did not have clinical disease. After progression to CH, the dogs in the miscellaneous group were treated with prednisone. The clinical outcomes are summarized in Table 5.

Survival Time

Survival after diagnosis was calculated for the most frequently observed forms of PH (Fig 1). Survival times are summarized in Table 5, also including the proportions of hepatitis-related deaths (events). No significant difference was found between AH and CH median survival time, but LDH was significantly lower than others ($P = .017$). Survival curves of both AH groups were not presented separately, owing to a low number of dogs and events in the AH(ca) group (n = 5). The 1 dog with *Leishmania*-induced CH was not included in the survival calculations, and was successfully treated with allopurinol, resulting in a survival time of 58 months at follow-up.

Prognostic Factors for Shortened Survival Time

A univariate Cox's proportional-hazard analysis (Table 6) was performed for AH, CH, and LDH (n = 95) to screen for variables with prognostic value for shortened survival time. Significant ($P < .10$) variables present at initial diagnosis are summarized in Table 6.

The multivariate analysis generated a model with prognostic factors of significant value for shortened survival

Table 5. Clinical outcome after therapy of PH at follow-up (n = 93; 2002–2006).

	AH(i)	AH(ca)	CH(i)	CH(ca)	LDH
No. of dogs with follow-up	15/16	5/5	43/43	23/24	7/7
Clinical outcome					
Remission (recurrence)	9 (2)	3 (0)	20 (9)	11 (5)	1 (1)
Residual disease	6	2	23	13	5
Alive at follow-up	6	3	16	7	0
Death (hepatitis related)	9 (7)	2 (2)	27 (24)	16 (13)	7 (7)
Survival time (*months*)					
EMST (95% CI)	34.9 (N/A)		18.3 (0.0–49.0)[a]	16.9 (6.8–27.0)	0.7 (0.4–1.0)
Mean (range)[b]	9.2 (0–35)		4.1 (0–30.6)	8.1 (0–36.8)	2.9 (0.1–11.8)

[a]Concerning n = 42 dogs, excluding the 1 dog with *Leishmania*-induced CH. Please note that 2 dogs were lost to follow-up;

[b]Concerning only dogs that died of hepatitis-related cause.

No., number; EMST, estimated median survival time (months); N/A, data not available; AH, acute hepatitis; CH, chronic hepatitis; i, idiopathic; ca, copper associated; LDH, lobular dissecting hepatitis; PH, primary hepatitis.

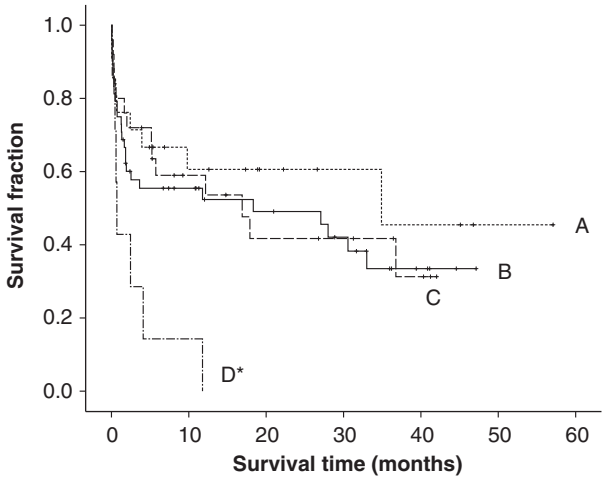

Fig 1. Kaplan-Meier curves for the survival time of dogs diagnosed with PH (n = 94; 2002–2006).

time. The variables that are individually associated with a shortened survival time in the analyzed population (n = 95) are presented in Table 7. Excluded were hypoproteinemia, hypoalbuminemia, prothrombin time (PT), activated partial thromboplastin time (APTT), and leukogram left shift, owing to missing data. Each of these initially excluded variables were singularly entered in new multivariate analyses, resulting in new models for shortened survival time. These additional models revealed that hypoalbuminemia ($P = .036$; HR 2.30; 95% CI 1.05–5.01) and a leukogram left shift ($P = .073$; HR 2.41; 95% CI 0.92–6.33) prognosticated a shortened survival time

Table 6. Significant prognostic factors ($P < .10$) for a shortened survival time in dogs with any form of PH (n = 95; 2002–2006), calculated with a univariate analysis (Cox regression).

Variable	No.	Events	*P*
Clinical signs			
Jaundice	95	53	.053
Lethargy	95	53	.007
Weight loss	95	53	.089
Abdominal distention	95	53	.013
Abdominal fluid wave	95	53	.007
Blood examination			
Hypoproteinemia	51	34	.017
Hypoalbuminemia	54	36	.005
PT (prolonged)	78	41	.004
APTT (prolonged)	78	41	.011
Leukogram left shift	47	33	.007
Ultrasonographic examination			
Small liver size	95	53	.077
Ascites	95	53	< .001
Enlarged portal lymph nodes	95	53	.069
Histopathologic examination			
Cirrhosis	95	53	.002

No, number; events, all dogs with hepatitis-related death; HR, hazard ratio; CI, confidence interval; PH, primary hepatitis; PT, prothrombin time; APTT, activated partial thromboplastin time.

Table 7. Significant prognostic factors for a shortened survival time in dogs with any form of PH (n = 95; 2002–2006), calculated with a "multivariate" analysis (Cox regression).

No.	Events	Variable Entered	Significant Variables	*P*	HR	95% CI
95	53	Jaundice	Jaundice	.013	2.19	1.18–4.06
		Lethargy	Abdominal fluid wave	.035	2.19	1.06–4.55
		Weight loss	Small liver size	.052	1.87	0.99–3.50
		Abdominal distention	Ascites	.006	2.43	1.28–4.59
		Abdominal fluid wave	Enlarged portal lymph nodes	.001	5.68	2.00–16.16
		Small liver size	Cirrhosis	.054	1.75	0.99–3.11
		Ascites				
		Enlarged portal lymph nodes				
		Cirrhosis				

No., number of dogs; events, all dogs with hepatitis-related death; HR, hazard ratio; CI, confidence interval; PH, primary hepatitis.

in these reduced populations in addition to the variables presented in Table 7.

Discussion

PH occurred in 0.5% of the canine referral population, with the highest prevalence seen for CH(i). Although we regularly encountered AH, the number of dogs referred may not reflect the true prevalence of the AH population in the Netherlands, because the majority of these dogs in our study group underwent spontaneous clinical remission either without therapy or with only short-term medical intervention, mostly resulting in long-term survival. In contrast, 5/12 of the pairwise-sampled AH dogs progressed toward CH, suggesting a considerable risk of the recurrence of clinical signs despite initial clinical remission. Administration of immunosuppressive medication, such as prednisone, before referral of the dogs with AH, may have stimulated progression to CH, or may have caused or compounded the deterioration of the acute condition.[2,10,36]

The majority of dogs with AH and CH were classified as idiopathic. Etiologic factors other than accumulation of copper were rarely found. Testing for infectious agents as a potential etiology was limited to serology (for leptospirosis) and histopathology. No specific tests were performed for detection of infectious agents with a possible etiologic role, because extensive investigations failed to detect any infectious agents in various forms of canine hepatitis.[2,10] No tests were performed for detection of liver-specific auto-antibodies. A few dogs (5/101) had concurrent diseases associated with immune aberrations; however, the existence of a causal relation with hepatitis is not established.[36] Retrospective staining of all liver histology slides with rubeanic acid revealed that

a remarkably large proportion (29/101) of all dogs had an increased hepatic copper concentration, suggesting a copper-associated etiology. The accumulation of copper does not automatically imply copper as the etiologic factor of hepatitis. However, it has recently been shown that copper is very unlikely to be an epiphenomenon of cholestasis when it is increased to >2 in the presently used grading system.[43] Furthermore, cholestasis causes periportal copper accumulation (zone 1) whereas all known forms of primary copper storage disease start with centrolobular accumulation (zone 3).[1] In all dogs in the AH(ca) and CH(ca) groups, copper staining did occur in zone 3 of the liver acini, and we suspect that copper may very likely have been the underlying cause of hepatitis in most of these dogs. This assumption is supported by their favorable response to D-penicillamine treatment. D-Penicillamine may also have anti-inflammatory effects that could also lead to clinical improvement independent of the effect on copper levels.[45–47] However, recent findings in copper-associated hepatitis in Labrador Retrievers demonstrate that D-penicillamine effectively decreases hepatic copper concentrations.[48] The authors recommend to routinely stain for copper with rubeanic acid and to grade copper semiquantitatively as standard procedures for liver histology in dogs.

AH and CH typically affected middle-aged to older dogs, with an on average earlier onset in copper-associated forms. The other groups were affected at a younger age. Female animals were much more frequently affected than males as reported earlier.[46] This suggests a female predisposition for both idiopathic and copper-associated forms of hepatitis. The breed composition of the study population differed from the overall clinic population, in accordance with earlier studies.[37,39,48] Most predisposed dog breeds were affected by idiopathic hepatitis. Labrador Retrievers and West Highland White Terriers have reportedly been associated with CH(ca),[34,42] but our results suggest that these breeds are also at increased risk for the idiopathic forms. A bias for breed disposition can be caused by increased awareness of a breeder association. Our previous studies into copper-associated hepatitis in Dobermann Pinchers[30,37,49] may have caused an underrepresentation in the current study, because most dogs have been included in another study and were therefore excluded from the investigated general hospital population.

The majority of dogs were presented with nonspecific clinical signs, which were also reported in earlier studies.[38,39] Sporadically, dogs were asymptomatic with only increased serum activity of liver enzymes and/or fasting bile acids. Generally, all PH groups had increased serum liver enzyme activity and/or fasting bile acids. Other frequently observed blood findings were hypoalbuminemia, elongated coagulation times (PT and APTT), decreased fibrinogen concentrations, and mild leukocytosis. Mean total serum protein was significantly lower in the LDH group when compared with the other groups, and mean serum glucose was significantly different between CH(i) and CH(ca). Neither the other mean blood values, nor the mean increased or decreased values of all blood measurements were significantly different between the studied groups. This could partly be the result of underpowered statistical testing. Ultrasonographic abnormalities, if present at all, were largely nonspecific. Our findings suggest that neither blood examination nor ultrasonography should be considered valid or reliable stand-alone diagnostic tests for hepatitis or for distinguishing between subtypes, with the exception of hepatic cirrhosis.

The examination of liver biopsy samples may have been subject to sampling errors, observer variation, and specimen size.[40,50] It is routine in our clinic to take at least 2 and preferably three 14 G liver biopsy samples of good quality for diagnostic purposes. We believe that sampling errors were an insignificant factor in this study; although they cannot be prevented altogether, our approach minimizes the incidence of such errors to a large extent. The WSAVA diagnostic criteria, however, had not been published at the time of 1st examination of our dogs. We, therefore, reevaluated all dogs by those criteria. Subsequently, the diagnoses of 29 dogs had to be amended, including 10 dogs with CH(ca), illustrating the clinical relevance of such criteria.

Owing to nonuniformity of the treatment in each group and small sample size in some groups, the EMST must be interpreted with care. Considerable overlap in confidence intervals and entanglement of survival curves occurred. However, the median AH survival time was much longer than that of both CH(i) and CH(ca). Dogs with severe AH (eg, fulminant hepatitis, n = 2) died during hospitalization, and LDH survival was significantly the shortest. It is likely that dogs with fulminant hepatitis and LDH do not live long enough to be referred and were underrepresented compared with given the true prevalence. No difference was found between CH(i) and CH(ca) groups in survival time, nor in mean age at presentation and prevalence of cirrhosis. This might be (partly) owing to the 10 initially misdiagnosed dogs with CH(ca) that, therefore, did not receive anticopper medication, which may have resulted in a shorter overall survival time in this subgroup. Regardless of the administered therapy, the EMSTs of both CH groups were comparable with those in 3 other retrospective studies. Two of these studies concerned mixed-breed populations, without distinguishing idiopathic and copper-associated etiology, and demonstrated a mean survival time of approximately 18 months (mean of n = 151, of which n = 58 received prednisone treatment)[37] and 16 months (mean of n = 57, range 1–87),[39] respectively. The 3rd study concerned a Labrador CH population, which also made no distinction between idiopathic and copper-associated cases, and found a survival time of approximately 1 year (median; range, 1 day–8 years).[38] We conclude that with better identification of copper-associated forms, considerable improvement of treatment effectiveness can be achieved for these forms of hepatitis. Until the etiologies underlying of idiopathic hepatitis have been elucidated, nonspecific regimens for the treatment of dogs with CH(i) need careful evaluation. However, improving therapeutic effectiveness may be reliant upon the identification of the relevant etiologies of these conditions.

The results of the multivariate analysis demonstrated that jaundice, abdominal fluid wave, hypoalbuminemia, leukogram left shift, microhepatica, ascites, enlarged

portal lymph nodes, and cirrhosis are prognostic factors for shortened survival time after diagnosis of PH. All of these findings are related to decompensated liver function and chronic portal hypertension. The majority of these prognostic factors occur in chronic forms of PH, thus explaining the observed differences in survival time between the PH groups. This is also demonstrated in previous retrospective studies into prognostic factors for shorter survival time. Their results are mostly in accordance with our findings, and include the following variables with a prognostic value: low serum glucose concentration and prolonged PT (death, <1 week), hypoalbuminemia and presence of bridging fibrosis (short-term survival, >1 week)[37]; anorexia, prolonged PT and APTT, and thrombocytopenia (short-term survival, <2 months)[38]; and low serum globulin concentration.[29]

This study underscores the clinical importance of the WSAVA diagnostic standards, which enables comparison of our results with those of other groups when the same standards are used.[1] We conclude that copper-associated hepatitis may be much more prevalent in the canine population than generally assumed and may account for approximately one third of all dogs with acute and chronic forms of PH. Furthermore, a considerable fraction of dogs with AH develop CH which may progress to cirrhosis. Signs of liver decompensation and cirrhosis at initial presentation are predictors of shortened survival time. Results from examination of prognostic factors in this study and previous studies suggest that early recognition and appropriate therapy are likely to be of key importance for prevention of survival-shortening disease progression. To current knowledge, this especially concerns copper-associated cases of hepatitis, because etiology-specific treatment is available. Further information on the etiology of idiopathic PH is likely necessary in order to prevent progression from AH to CH to cirrhosis by application of etiology-specific treatment.

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Footnote

[a] SPSS version 15.0, Benelux BV, Gorinchem, the Netherlands

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