Journal of Veterinary Internal Medicine
Open Access

J Vet Intern Med 2015;29:644–650

Serum Bile Acid Concentrations, Histopathological Features, and Short-, and Long-term Survival in Horses with Hepatic Disease

B. Dunkel, S.A. Jones, M.J. Pinilla, and A.K. Foote

Background: Serum bile acid concentrations (SBA) and a histopathological biopsy score [*Equine Vet J* 35 (2003) 534] are used prognostically in equine hepatic disease.

Hypothesis: Histopathologic features and scores, but not SBA, differ between survivors and nonsurvivors and correlate with histopathologic evidence of hepatic inflammation and fibrosis.

Animals: Retrospective study. Records (1999–2011) of horses with hepatic disease diagnosed by biopsy and with concurrent measurements of SBA.

Methods: Retrospective cohort study. Biopsies were examined for inflammatory cell infiltration including type and distribution, fibrosis, irreversible cytopathology affecting hepatocytes, hemosiderin, or other pigment deposition and bile duct proliferation. SBA, histopathological findings and a histological score [*Equine Vet J* 35 (2003) 534] were compared between short- (survival to discharge) and long-term (>6 months) survivors and correlations between SBA and histopathological findings investigated.

Results: Of 81 cases 90% survived short-term and 83% long-term. Short-term and long-term nonsurvival were associated with SBA ($P = .009$; $P = .006$), overall ($P = .001$; $P = .002$) and parenchymal (short-term only; $P = .01$) inflammation, portal and bridging fibrosis (all $P < .001$), apoptosis or single cell necrosis ($P < .001$; $P = .008$), hemosiderin deposition in hepatocytes ($P = .011$; $P = .028$), biliary (both $P < .001$), vascular ($P = .003$; $P = .045$) and endothelial ($P < .001$; $P = .02$) hyperplasia, nucleic changes ($P = .004$; $P < .001$) and the histopathological score (both $P < .001$). SBA were significantly and positively correlated with overall ($P = .001$), parenchymal ($P < .001$) and portal ($P = .004$) inflammation and portal ($P = .036$) and bridging ($P = .002$) fibrosis.

Conclusions and Clinical Importance: SBA, histopathological findings and scores differ between survivors and nonsurvivors. SBA concentrations are associated with inflammation and fibrosis suggesting interference with hepatic function. A histopathological score >2 and, less so, SBA >20 μmol/L are specific but not sensitive indicators of nonsurvival.

Key words: Hepathopathy; Hepatic failure; Hepatitis; Liver biopsy.

Currently, histopathologic examination of liver tissue is regarded by many clinicians as the most sensitive method of diagnosing hepatic disease[1,2] and histopathological findings from biopsied tissue in people correlate well with subsequent gross postmortem findings.[3] Histopathology is also regarded as the best indicator of prognosis in hepatic disease in horses and a scoring system has been developed for this purpose.[4] However, this scoring system has not been evaluated in a different case population. In the absence of a liver biopsy or for continuous monitoring purposes, many clinicians anecdotally use serum bile acid concentrations (SBA) as a surrogate prognostic indicator and concentrations exceeding 20 μmol/L are predictive of nonsurvival.[5]

Abbreviations:

SBA	serum bile acid concentrations

However, our clinical impression indicated that this might not be uniformly applicable to all equine populations with hepatic disease. The prognostic value of SBA relies on the fact that the detected loss of hepatic function is permanent or progressive or both. It is conceivable that reversible changes, such as inflammatory infiltration or reversible hepatocyte damage, could temporarily interfere with hepatic function, leading to an increase in SBA. With appropriate treatment or time, the condition might resolve which would make SBA an unreliable prognostic indicator. Furthermore, a large number of horses in the early stages of liver disease have SBA within the reference ranges[6] and gaining additional prognostic information from histopathologic features would be useful. To date, few studies have investigated the relationship between SBA and individual histopathologic features and short- and long-term survival. As SBA are considered to be indicators of liver function, a close association between SBA and histopathological findings would be expected, particularly between histological findings such as inflammation and fibrosis, that could interfere with normal function.

The aim of the study was to determine whether SBA and histologic variables including a histological score were associated with short- (survival to discharge) and long-term (>6 months after discharge) survival; differences between biochemical and hematological variables

From the Department of Clinical Science and Services, Royal Veterinary College, North Mymms, UK (Dunkel, Jones); the Animal Health Trust, Newmarket, UK (Pinilla); and the Beaufort Cottage Laboratories, Rossdale and Partners, Newmarket, UK (Foote).

Parts of the study were presented as an abstract at the European College of Equine Internal Medicine Congress in Hannover 2011.

Corresponding author: Bettina Dunkel, Department of Clinical Science and Services, The Royal Veterinary College, North Mymms, Hertfordshire AL9 7TA, UK; e-mail: bdunkel@rvc.ac.uk.

Submitted August 5, 2014; Revised November 20, 2014; Accepted January 15, 2015.

DOI: 10.1111/jvim.12551

in short- and long-term survivors and nonsurvivors were also evaluated. The study further assessed whether SBA concentrations in horses with liver disease correlated with histologic features. It was hypothesized that histopathologic features and score, but not SBA, differed between survivors and nonsurvivors and that SBA concentrations correlated with histopathologic evidence of inflammation and fibrosis.

Materials and Methods

The study was carried out in accordance with the ethical guidelines of the participating institutions. In a retrospective study, all horses presenting to two equine referral hospitals that had a liver biopsy and concurrent (± 3 days) SBA measurement performed between the years 1999 and 2011 were eligible for inclusion into the study. The animal's date of birth, age at biopsy, gender and breed, SBA and, where available, other clinicopathological data were recorded.

Archived formalin-fixed paraffin wax embedded trucut liver biopsy samples were recut and 4 μm sections stained with haematoxylin & eosin and van Gieson staining. To highlight fibrosis, deparaffinised sections were stained for 7 minutes with Van Gieson stain (picric acid and aqueous acid fuchsin, Leica Biosystems, Leica Microsystems (UK), Ltd, Milton Keynes, Buckinghamshire, UK), dehydrated through alcohol, cleared in xylene and mounted with DPX. All samples were reviewed and graded by two pathologists (MJP and AKF) blinded to the outcome of the case. Only specimens with more than 6 lobules were scored. The portal tracts and hepatic parenchyma were assessed for bile duct proliferation, fibrosis, irreversible cytopathology affecting hepatocytes, hemosiderin or other pigment deposition and inflammatory cell infiltration as detailed below.

The total length of the biopsies or where more than one slice was available, the sum of the lengths was documented. Portal numbers were counted and the degree of inflammatory infiltration overall and separately within portals and parenchyma was scored as absent, mild, moderate or severe. Inflammatory infiltrates were graded as mild if up to 5 leucocytes were present within a portal tract or lobule, moderate if 5–20 leucocytes were seen and severe if more than 20 leucocytes were found.

Cell types within portal areas were counted and described based on the following protocol: 0 = no inflammatory cells present, 1 = mononuclear cells present alone, 2 = neutrophilic with or without mononuclear cell infiltrates and 3 = hemosiderophages alone, or in combination with any other cell types. Cell types within the parenchyma were also identified and described: 0 = no inflammation, 1 = neutrophilic infiltration in the parenchyma and also affecting portal areas, 2 = neutrophilic infiltration without portal infiltrates and 3 = other cellular infiltrate.

Fibrosis was assessed and classified as absent, mild, moderate or severe. Fibrosis was defined as mild where immature or mature fibrous tissue expanded a portal tract up to twice the normal size, moderate where a tract was 3 times the normal size and severe where a tract was greater than or equal to 4 times the normal size (often seen as bridging fibrosis across adjacent lobules). Bridging fibrosis was also further graded as absent, mild (delicate fibrosis confined to portal-portal distribution), moderate (extensive fibrosis but still predominantly confined to portal-portal regions) or severe (extensive fibrosis, extending across the portal plate, with disruption of the normal lobular pattern).

Apoptosis or single cell necrosis or both were graded as absent, mild (less than 2 cell per lobule), moderate (2–5 cells per lobule) or severe (>5 cells per lobule, often also including some individual cell necrosis).

Distribution of hemosiderin was assessed within 3 regions: within Kupffer cells, portal areas and hepatocytes. Presence of hemosiderin was recorded as absent, mild (fewer than 25% of cells affected), moderate (25–50% of cells affected) and severe (>50% of cells affected).

Bile duct proliferation, vascular hyperplasia and hyperplasia of the sinusoidal endothelium (endothelial hyperplasia) were graded according to the number of biliary branches/number of blood vessels/hyperplastic sections in portal tracts sectioned in a typical portal triad as absent or mild containing 2–3, moderate containing 4–6 and severe containing greater than 7 biliary branches/vessels/sections.

Bile stasis (canalicular and ductal) was assessed as absent or present.

Cytoplasmic swelling was also assessed and scored as absent, mild (approximately 1.5× normal diameter), moderate (approximately 2× normal diameter) or severe (≥3× normal diameter). The swelling distribution was then further classified as absent, affecting hepatocytes closest to arterial and portal inflow, within the transitional zone, periacinar (comprising the hepatocytes nearest to the outflow; terminal hepatic venule), or combinations of the above.

Changes to hepatocyte nuclei (nucleic changes) were scored as absent (no significant anisokaryosis), mild (mild anisokaryosis without megalocytosis), moderate (moderate anisokaryosis ± occasional megalocytosis) and severe (frequent megalocytosis >1 per lobule).

A histological score was assigned.[4]

Records were reviewed to determine if animals were known to have died, either before discharge or subsequently. Owners or the referring veterinary surgeons of horses that were not known to be dead were contacted at least 6 months after discharge to determine survival outcome. Where applicable, the date and cause of death was ascertained. Survival was categorised as short- (survival to discharge) and long-term survival (survival to at least 6 months after discharge).

Statistical analysis

Continuous data are summarized as mean ± standard deviation (normally distributed), median (range) (not normally distributed) and categorical data as number and percentage. Normality of the data was assessed using a Shapiro-Wilk test. Differences in SBA, histological features and scores and biochemical and hematologic variables between short- and long-term survivors and nonsurvivors were explored using a Student's *t*-test (normally distributed continuous data) or Mann-Whitney *U*-test (not normally distributed continuous data) and chi-square or Fisher's exact test (categorical data), respectively. The correlation between log transformed SBA and histological features was explored using univariate analysis of variance. Receiver-operator curves were generated to determine optimal cut-off points for SBA as indicator for short- and long-term nonsurvival and histopathology scores as indicators for short- and long-term nonsurvival. Sensitivity and specificity were reported. In addition, the previously suggested cut-off point of >20 μmol/L was explored in the same manner. The area under the curve (AUC) was also determined. All statistical analyses were performed using a commercially available software program[a] ; significance was set at $P \leq .05$.

Results

Eighty-one horses fulfilled the inclusion criteria for the study, 36 from hospital 1 and 45 from hospital 2; 24 horses (30%) were female and 57 (70%) were male with a mean age of 11 ± 5.4 years. Two horses from hospital 1 had biopsies performed before June 2001 and might have been included in an earlier study.[4] Eight

(10%) horses died or were euthanized before discharge from the hospital whilst 6 (7%) animals were euthanized or died within 6 months of discharge, all for reasons pertaining directly to liver disease. Sixty-seven animals (83%) survived long-term and 14 (17%) did not.

The mean biopsy length was 18.4 ± 7.8 mm and was not different between short-term survivors and nonsurvivors (18.5 ± 7.9 mm versus 16.8 ± 8.2 mm; $P = .58$) and long-term survivors and nonsurvivors (18.2 ± 7.8 mm versus 18.9 ± 8.6 mm; $P = .76$). SBA, overall and parenchymal (short-term survival only) inflammation, portal and bridging fibrosis, apoptosis or single cell necrosis, or both hemosiderin deposition in hepatocytes, biliary and vascular hyperplasia, endothelial hyperplasia, nucleic changes, and the histopathology score were significantly different between survivors and nonsurvivors (Tables 1–3). Comparison of biochemical and hematologic variables between short- and long-term survivors and nonsurvivors are shown in Table 1.

SBA were significantly and positively correlated with overall, parenchymal and portal inflammation, portal and bridging fibrosis, hemosiderin deposition in Kupffer cells, nucleic changes and the histological score (Table 4).

The AUC for SBA was 0.78 (95% confidence interval [CI]: 0.66–0.91; $P = .009$) for short-term survival and 0.8 (95% CI: 0.6–1.0; $P = .005$) for long-term survival. The AUC for the histological score was 0.74 (95% CI: 0.56–0.89; $P = .006$) as indicator for short-term survival and 0.85 (95% CI: 0.7–0.99; $P < .001$) as indicator for long-term nonsurvival. The optimal cut off points for short- and long-term nonsurvival were SBA ≥17 μmol/L and a histology score >3 and ≥16 μmol/L and a histological score >2, respectively. Sensitivity and specificity are reported in Table 5.

No long-term survivor had a score >3. However, 29% (n = 4) of horses that did not survive >6 months had a score of 0 or 1 and SBA concentrations of 2 of these (14%) were also within the reference range (<12.8 μmol/L).

Discussion

In agreement with other studies SBA concentrations were higher in short- and long-term nonsurvivors than short- and long-term survivors.[5,7] However, the clinical impression that high SBA were not necessarily associated with nonsurvival in the examined population was supported by the relatively low specificity of two cut-off points (one determined in the study and the previously reported cut-off of ≥20 μmo/L).

The diagnostic value of SBA in people is undisputed as an increase in the serum is a highly specific for hepatic disease, although only moderately sensitive. In cases of liver cirrhosis in people SBA have long-term prognostic value[8,9] while in cases of acute hepatitis, clinical improvement is often paralleled by decreasing SBA, corresponding with normalizing hepatic function.[10,11] In these instances, one time measurements of SBA would not be useful prognostic indicators.

In horses, classically described hepatic diseases such as serum hepatitis, cholangioheptitis, neoplasia, *Clostridium piliformis* infection, and pyrrolizidine alkaloid toxicity are all associated with severe or irreversible liver damage, or both, and a high case fatality, often ranging from 50 to 100%.[7,12,13] In many of these cases, loss of hepatic function is expected to be permanent or progressive, or both, and SBA are likely to be indicative of extend of the irreparable damage and therefore also of the prognosis. In these cases, a close association between SBA, presence and severity of hepatic damage and a negative outcome would be expected. Similar to the findings in people, SBA would be expected to correlate with irreversible hepatic changes. In this study, a correlation between SBA and portal and bridging fibrosis as well as nucleic changes, which are considered to be irreversible, was demonstrated and those histological features were also more commonly observed in nonsurvivors.

However, SBA returned to normal limits in horses that survived hepatic necrosis which presumably corresponded to improved hepatic function as regeneration ensued.[14] The horses examined here experienced predominately mild-to-moderate hepatic disease of unknown etiology and in these cases SBA might be more indicative of a temporary and potentially reversible compromise of hepatic function. This assumption is supported by the low specificity of SBA >17 and >20 μmol/L for nonsurvival and the finding of significant correlation between SBA and potentially reversible histological findings such as inflammation and hemosiderin accumulation in Kupffer cells. However, overall and parenchymal (short-term survival only), but not portal, inflammation was also more common in nonsurvivors and although the inflammation may be reversible, it could equally be progressive. Enhanced prognostic information could therefore probably be gained from serial SBA monitoring.[14]

The histological score performed well as a specific albeit only moderately sensitive indicator of short- and long-term nonsurvival.[4] Based on the high specificity the histological score provided the best prognostic information for long-term survival and was numerically superior to SBA for short- and long-term survival. A limitation of the study is that the score was developed in the same geographic location (South-East England) in which this study was performed and it is likely that the nature of hepatic disease was very similar in the horses used to develop the score to the horses investigated here. Results might not be directly transferrable to areas with very different disease types and re-evaluation of the scoring system is therefore advisable before it is used for prognostic purposes in different areas. Furthermore, all horses with hepatic disease were analysed together, irrespective of the underlying etiology and results might be different for certain subpopulations. The scoring system was designed for diffuse, non-neoplastic hepatopathies and most cases described here would have fallen into this category.[4] Lastly, outcome could have been influenced by the initiation of adequate treatment, or lack thereof. All nonsurvivors in the study

Table 1. Comparison of biochemical and haematological variables between short- (survival to discharge) and long-term (survival >6 months after discharge) survivors and nonsurvivors.

	Short-term Survivor	Short-term Nonsurvivor	*P*-value	Long-term Survivor	Long-term Nonsurvivor (All Nonsurvivors[a])	*P*-value
Serum bile acids (μmol/L)	12.5 (1.5–82.5) n = 73	28.1* (11.9–42.8) n = 8	.009	12.3 (1.5–82.5) n = 67	28.1* (6.3–45.2) n = 14	.006
Total plasma protein (g/dL)	6.5 ± 0.6 n = 68	7.5 ± 1.1* n = 6	.001	6.5 ± 0.58 n = 62	6.9 ± 1.0 n = 12	.094
Albumin (g/dL)	3.5 ± 0.41 n = 69	2.8 ± 0.71* n = 6	.003	3.5 ± 0.38 n = 63	2.9 ± 0.48* n = 12	<.001
Globulin concentration (g/dL)	3.0 (1.8–4.4) n = 68	4.2* (4.0–7.2) n = 6	<.001	3.0 (1.8–4.1) n = 62	4.0* (2.1–7.2) n = 12	.001
Fibrinogen (mg/dL)	280 (100–950) n = 57	690* (180–730) n = 5	.025	270 (100–510) n = 52	590* (100–950) n = 10	.019
AST (IU/L)	530 (227–2520) n = 72	482 (159–651) n = 5	.26	547 (227–2520) n = 66	436 (159–762) n = 11	.091
Creatine kinase (IU/L)	300 (86–2180) n = 64	336 (179–2889) n = 5	.43	306 (86–2180) n = 58	277 (179–2889) n = 11	.84
LDH (IU/L)	730 ± 305 n = 30	393 ± 379 n = 3	.13	641 (318–1614) n = 29	279* (171–831) n = 4	.041
Sorbitol dehydrogenase (IU/L)	16 (1.9–209) n = 36	181 (25–328) n = 3	.051	17 (1.9–209) n = 31	22 (8.5–328) n = 8	.59
Gamma-glutamyltransferase (IU/L)	130 (14–1587) n = 73	121 (49–185) n = 3	.56	136 (14–1587) n = 67	90 (42–300) n = 9	.24
GLDH (IU/L)	7.9 (1.2–5704) n = 31	11 (9–12.7) n = 2	.85	7.2 (1.2–5704) n = 30	12.7 (9.2–15.3) n = 3	.68
Urea (mg/dL)	14.3 (6.4–22.4) n = 49	16 (10.1–56.9) n = 5	.32	14.6 (9.8–22.4) n = 45	13.7 (6.4–56.9) n = 9	.51
Creatinine (mg/dL)	1.3 (0.9–2.1) n = 62	1.2 (1.1–4.4) n = 5	.21	1.4 (0.9–2.1) n = 56	1.2 (1.1–4.4) n = 11	.1
Serum amyloid A (mg/L)	0 (0–249) n = 38	147* (0.6–990) n = 4	<.001	0 (0–67.3) n = 36	147* (0.6–990) n = 6	<.001
Total bilirubin (mg/dL)	1.5 (0.4–12.3) n = 57	4.4* (2.5–6.1) n = 3	.023	1.4 (0.4–12.3) n = 51	2.5* (0.9–6.1) n = 9	.027
Direct bilirubin (mg/dL)	0.25 ± 0.09 n = 20	0.4 n = 1		0.2 (0.1–0.4) n = 19	0.2 and 0.4 n = 2	.4
Indirect bilirubin (mg/dL)	1.2 (0.2–6.8) n = 20	5.9 n = 1		1.2 (0.2–6.8) n = 19	1.5 and 5.9 n = 2	.23
Triglycerides (mg/dL)	41 (15–743) n = 8	1496 (32–3186) n = 3	.15	41 (15–743) n = 8	1496 (15–3186) n = 3	.15
Red blood cell count ($\times 10^6/\mu L$)	7.9 (4.7–11.8) n = 31	10.0 (7.8–17.8) n = 4	.097	8.0 (4.7–11.8) n = 29	8.5 (5.2–17.8) n = 5	.45
Hemoglobin (g/dL)	13.2 (8.9–17.2) n = 31	11.5 (7.6–16.4) n = 4	.64	13.3 (9.9–17.2) n = 30	8.9 (7.6–16.4) n = 5	.26
Leukocyte count ($\times 10^3/\mu L$)	7.5 (3.3–18.1) n = 60	8.1 (4.2–14.3) n = 5	.52	7.3 (3.3–18.1) n = 54	8.1 (4.2–16.4) n = 11	.077
Neutrophil count ($\times 10^3/\mu L$)	4.2 (1.4–14.9) n = 59	7.0 (1.2–11.5) n = 4	.32	4.1 (1.4–14.8) n = 53	7.0* (1.2–13.3) n = 10	.042
Lymphocyte count ($\times 10^3/\mu L$)	2.5 ± 0.9 n = 59	2.3 ± 0.7 n = 4	.71	2.5 ± 0.9 n = 53	2.7 (1.1–4.6) n = 10	.55

[a]Long-term nonsurvivors include all short- and long-term nonsurvivors.

*$P \leq .05$.

AST, aspartate aminotransferase; LDH, lactate dehydrogenase; GLDH, glutamate dehydrogenase; statistical significance was set at $P \leq .05$.

were euthanized because of progression of hepatic disease, despite administration of treatment, and no animal was euthanized because of financial limitations.

In the addition to the histological features evaluated in the previous study,[4] other histologic aspects of hepatic disease were included that, to the authors' knowledge, have not been investigated previously. Endothelial hyperplasia, a feature in people referred to as capillarization of the sinusoidal endothelium, is defined as the loss of the characteristic fenestrated phenotype of the endothelial cells with formation of an organized basement membrane. Capillarization precedes fibrosis in people and experimental animals and it has been suggested that capillarization might even initiates the

Table 2. Comparison of histopathological findings between short- (survival to discharge; A) and long-term (survival >6 months after discharge; B) survivors and nonsurvivors.

	Short-term Survivor (n = 73) (% Horses)				Short-term Nonsurvivor (n = 8) (% Horses)				
(A)	Absent	Mild	Moderate	Severe	Absent	Mild	Moderate	Severe	*P*-value
Inflammation*	4	63	32	1	0	50	25	25	.009
Portal inflammation	6	63	27	4	13	50	13	25	.09
Parenchymal inflammation*	40	44	15	1	25	38	13	25	.01
Portal fibrosis*	13	67	8	3	13	0	63	25	<.001
Bridging fibrosis*	59	36	6	0	13	25	50	13	<.001
Apoptosis, single cell necrosis, or both*	70	26	3	0	50	25	0	25	<.001
Hemosiderin (portal)	48	37	14	1	38	38	13	13	.29
Hemosiderin (Kupffer cells)	44	48	7	1	38	38	13	13	.24
Hemosiderin (hepatocytes)*	73	21	7	0	75	0	13	13	.011
Biliary hyperplasia*	71	25	4	0	38	38	0	25	<.001
Vascular hyperplasia*	61	37	1	0	50	25	25	0	.003
Bile stasis	100	0	0	0	88	13	0	0	.099
Endothelial hyperplasia*	78	22	0	0	25	63	13	0	<.001
Cytoplasmatic swelling	27	55	18	1	38	50	13	0	.9
Distribution of cytopl swell[b]									.9
Nucleic changes*	49	49	1	0	38	38	25	0	.004
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**	
Portal cell types	6	41	18	34	12.3	25	13	50	.65
Parenchymal cell types	40	27	23	10	25	38	13	25	.46

	Long-term Survivor (n = 67) (% Horses)				Long-term Nonsurvivor (All Nonsurvivors[a]; n = 14) (% Horses)				
(B)	Absent	Mild	Moderate	Severe	Absent	Mild	Moderate	Severe	*P*-value
Inflammation*	5	64	31	0	0	50	29	21	.002
Portal inflammation	6	64	27	3	7	50	21	21	.075
Parenchymal inflammation	40	45	13	2	29	36	21	14	.1
Portal fibrosis*	22	73	5	0	14	0	57	29	<.001
Bridging fibrosis*	61	36	3	0	21	29	43	7	<.001
Apoptosis, single cell necrosis or both*	72	25	2	0	50	29	7	14	.008
Hemosiderin (portal)	51	37	10	2	29	36	29	7	.14
Hemosiderin (Kupffer cells)	45	46	8	2	36	50	7	7	.63
Hemosiderin (hepatocytes)*	72	22	6	0	79	0	14	7	.028
Biliary hyperplasia*	75	24	2	0	36	36	14	14	<.001
Vascular hyperplasia*	64	34	2	0	43	43	14	0	.045
Bile stasis	100	0	0	0	93	7	0	0	.173
Endothelial hyperplasia*	78	22	0	0	50	43	7	0	.02
Cytoplasmatic swelling	30	54	16	0	21	57	14	7	.16
Distribution of cytopl swell[b]									.75
Nucleic changes*	52	48	0	0	29	50	21	0	<.001
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**	
Portal cell types	6	42	15	36	7	29	29	36	.62
Parenchymal cell types	40	25	25	9	29	43	7	21	.17

[a]Long-term nonsurvivors include all short- and long-term nonsurvivors.

[b]Cytoplasmatic swelling was assessed in 5 distribution patterns, a detailed description of the grading criteria used is given in the text and only the *P*-value is displayed.

*$P \leq .05$; statistical significance was set at $P \leq .05$.

Different inflammatory cell infiltrates are displayed in bold text.

process.[15] Interestingly, endothelial hyperplasia was significantly associated with short- and long-term survival in this study but further investigations are required to investigate the importance and implications of this histological finding.

Similar to other studies[5,7,14] significant differences in haematological and biochemical data between survivors and nonsurvivors were established. As previously reported,[5] low albumin and high globulin concentrations were associated with short- and long-term nonsurvival. Low albumin concentrations are an infrequent finding in hepatic disease in horses.[16] The presence of hypoalbuminemia in combination with hyperglobulinemia might therefore be particularly relevant when trying to establish a prognosis. The finding of lower LDH activities in nonsurvivors was unexpected and is difficult to explain. LDH is not a liver-specific enzyme and it is possible that muscle damage

Table 3. Comparison of histological score as described by Durham et al. (2003) between short- (survival to discharge; A) and long-term (survival >6 months after discharge; B) survivors and nonsurvivors.

(A)	Short Term Survivor (n = 73)						Short-term Nonsurvivor (n = 8)					*P*-value
Histological score	0	1	2	3	4	5	0	1	4	8	14	
	52 (71%)	12 (16%)	2 (3%)	5 (7%)	1 (1%)	1 (1%)	2 (25%)	1 (13%)	3 (38%)	1 (13%)	1 (13%)	<.001

(B)	Long-term Survivor (n = 67)				Long-term Nonsurvivor (All Nonsurvivors[a]; n = 14)								*P*-value
Histological score	0	1	2	3	0	1	2	3	4	5	8	14	
	51 (78%)	12 (18%)	1 (2%)	3 (5%)	3 (21%)	1 (7%)	1 (7%)	2 (14%)	4 (29%)	1 (7%)	1 (7%)	1 (7%)	<.001

[a]Long-term nonsurvivors include all short- and long-term nonsurvivors.
*$P \leq .05$; statistical significance was set at $P \leq .05$.

Table 4. Correlation between log transformed serum bile acid concentrations and histological features and scores.

	Regression Coefficient ± SEM	*P*-value
Inflammation*	0.18 ± 0.051	.001
Portal inflammation*	0.141 ± 0.047	.004
Parenchymal inflammation*	0.155 ± 0.038	<.001
Portal fibrosis*	0.095 ± 0.045	.036
Bridging fibrosis*	0.141 ± 0.044	.002
Apoptosis and/or single cell necrosis	0.085 ± 0.051	.099
Hemosiderin (portal)	0.083 ± 0.042	.051
Hemosiderin (Kupffer cells)*	0.104 ± 0.046	.026
Hemosiderin (hepatocytes)	0.053 ± 0.05	.291
Biliary hyperplasia	0.094 ± 0.049	.056
Vascular hyperplasia	0.056 ± 0.06	.347
Bile stasis	0.078 ± 0.306	.8
Endothelial hyperplasia	0.068 ± 0.07	.33
Cytoplasmatic swelling	−0.01 ± 0.049	.85
Distribution of cytopl swell	0.004 ± 0.011	.69
Nuclei*	0.199 ± 0.055	.001
Histological score*	0.05 ± 0.015	.002

*$P \leq .05$.
Distribution of cytopl. swell: distribution of cytoplasmatic swelling; SEM: standard error of the mean; statistical significance was set at $P \leq .05$.

Table 5. Sensitivity and specificity of different cut-off points for serum bile acid concentrations (SBA) and histological score as indicators of nonsurvival.

Short-term Nonsurvival	Sensitivity (%)	Specificity (%)
SBA ≥17 μmo/L	75	70
SBA ≥20 μmo/L	62.5	78.1
Histological score >2	62.5	90.4
Histological score >3	62.5	97.3
Long-term nonsurvival (all nonsurvivors[a])		
SBA ≥16 μmo/L	78.6	65.7
SBA ≥20 μmo/L	57.1	80.6
Histological score >2	64.3	95.5
Histological score >3	50	100

[a]Long-term nonsurvivors include all short- and long-term nonsurvivors.

in survivors in association with the low number of nonsurvivors in which the test was performed has contributed to the finding of this counterintuitive statistical difference although differences in creatine kinase (CK) might have been expected if muscle damage was involved. Plasma fibrinogen concentrations are higher in nonsurviving horses with hepatic disease and it is possible that long-standing inflammatory processes in the liver ultimately lead to liver failure and death.[5] No specific inflammatory cell type was associated with nonsurvival. While parenchymal and portal inflammation correlate with SBA and therefore might negatively influence liver function only parenchymal inflammation was associated with short-term survival. This could suggest that parenchymal inflammation is more likely to contribute to or trigger events that lead to hepatic failure and nonsurvival. Further large scale investigation into the different distributions and also types of inflammatory infiltrates are clearly needed.

In summary, SBA, some histopathological findings and histopathological scores differed between survivors and nonsurvivors. SBA concentrations are associated with inflammation and fibrosis suggesting that both interfere with hepatic function. A histopathological score >2 and, to a lesser degree, SBA >20 μmol/L are specific but not sensitive indicators of nonsurvival. For the population examined the histological score offered the best specificity for long-term survival.

Footnote

[a] IBM SPSS statistics 19, P.O. Box 41, North Harbour Portsmouth, Hampshire PO6 3AU, UK

Acknowledgments

Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Pearson EG. Liver disease in the mature horse. Equine Vet Educ 1999;11:87–96.

2. Durham AE, Smith KC, Newton JR. An evaluation of diagnostic data in comparison to the results of liver biopsies in mature horses. Equine Vet J 2003;35:554–559.

3. Giesen CP, Koepsell JE, Hastings EV, et al. Correlation of punch liver biopsy with autopsy material. Am J Dig Dis 1951;18:304–307.

4. Durham AE, Smitht KC, Newton JR, et al. Development and application of a scoring system for prognostic evaluation of equine liver biopsies. Equine Vet J 2003;35:534–540.

5. Durham AE, Newton JR, Smith KC, et al. Retrospective analysis of historical, clinical, ultrasonographic, serum biochemical and haematological data in prognostic evaluation of equine liver disease. Equine Vet J 2003;35:542–547.

6. Pearson EG, Craig AM. The diagnosis of liver disease in equine and food animals. Mod Vet Pract 1980;61:233–237.

7. McGorum BC, Murphy D, Love S, et al. Clinicopathological features of equine primary hepatic disease: A review of 50 cases. Vet Rec 1999;145:134–139.

8. Mannes GA, Thieme C, Stellaard F, et al. Prognostic significance of serum bile acids in cirrhosis. Hepatology 1986;6:50–53.

9. Siciliano M, Barbesino G, Marra L, et al. Long-term prognostic value of serum bile acids in liver cirrhosis: A prospective study. Z Gastroenterol 1989;27:653–656.

10. Tobiasson P, Forkman A. Serum bile acids in acute hepatitis. Scand J Gastroenterol 1981;16:145–149.

11. Marchettini G, Matergi M, Chirone E, et al. [Serum bile acids in acute hepatitis. Clinical observations]. Quad Sclavo Diagn 1980;16:214–226.

12. Guglick MA, MacAllister CG, Ely RW, et al. Hepatic disease associated with administration of tetanus antitoxin in eight horses. J Am Vet Med Assoc 1995;206:1737–1740.

13. Johnston JK, Divers TJ, Reef VB, et al. Cholelithiasis in horses: Ten cases (1982-1986). J Am Vet Med Assoc 1989;194:405–409.

14. West HJ. Clinical and pathological studies in horses with hepatic disease. Equine Vet J 1996;28:146–156.

15. DeLeve LD. Hepatic microvasculature in liver injury. Semin Liver Dis 2007;27:390–400.

16. Parraga ME, Carlson GP, Thurmond M. Serum protein concentrations in horses with severe liver disease: A retrospective study and review of the literature. J Vet Intern Med 1995;9:154–161.