Hepatic Enzyme Profile in Horses

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For diagnostic purposes, liver enzymes are usually classified into hepatocellular and cholestatic. These two groups of equine liver-specific enzymes include sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GLDH), γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP). SDH and GLDH mostly reflect hepatocellular injury and cholestasis, while GGT expresses high values in biliary necrosis or hyperplasia. Likewise, AST, LDH, and ALP also reflect hepatocellular and biliary disease, but these enzymes are not liver specific. From the clinical point of view of the course of liver or biliary disease, AST and ALP are indicative of chronic disease, whereas SDH, GGT, and GLDH indicate an acute course.

Keywords: hepatobiliary disease ; liver ; enzyme profile ; horse

1. Introduction

Within the concept of liver disease, researchers can include different pathologies that directly or indirectly affect liver function. In turn, these alterations in liver function may be temporary or may progress to complete and irreversible failure [1]. Although liver failure is rare in equines, different authors point out that the clinical manifestation occurs when more than 70% of the function of this organ is lost [1][2][3][4]. Liver disease and liver failure are two distinct and important concepts that must be differentiated. Among all liver functions, the reserve is extremely important. Therefore, a loss of at least 60–80% of the mass of this organ is required to show clear signs of liver failure. This clarification becomes important because some indicators of liver function, such as albumin or coagulation times, are affected when this organ is insufficient and are therefore not sensitive for detecting disease in early stages [2][3][5].

Several different etiological causes may be ascribed to the development of horse hepatic diseases [1]. Pre-existing sepsis, hypoxia, neoplasia, toxic or metabolic causes in both foals and adult horses can lead to liver damage [1][6][7][8]. The consumption of toxic plants (e.g., mugwort and clover) and mycotoxins (e.g., aflatoxin, zearalenone, and fumonisin) also can develop hepatic disease [9][10][11][12].

Infectious and non-infectious causes of liver and biliary tract inflammation have also been described. These include serum hepatitis or Theiler's disease or viral hepatitis (caused by equine herpesvirus, hepatitis, equine hepacivirus, and equine parvovirus), parasitic hepatitis (caused by large strongyles and ascarids), Tyzzer's disease (Clostridium piliforme), inflammatory diseases such as cholangiohepatitis (due to cholelithiasis or intestinal obstruction), displacement of the right dorsal colon with bile duct obstruction, cholelithiasis, hepatic torsion, portal vein thrombosis and hyperlipemia [8][13][14][15][16][17]. Other causes of liver disease are primary neoplasms such as hepatocellular carcinoma, cholangiocarcinoma, or hepatoblastoma. Additionally, metastatic dissemination of lymphomas or malignant melanomas to the liver from other primary locations [8][18]. The main analytes to evaluate in blood samples related to liver disease are serum enzymes and indicators of residual liver functional capacity [6]. In general terms, researchers can classify liver enzymes into two main groups. On the one hand, some are filtered from the cytoplasm of the damaged hepatocyte called hepatocellular, and on the other hand, those that increase their concentration in the blood due to an increased synthesis as a response to the decrease or absence of normal bile flow from the liver to the duodenum also called cholestasis. In hepatocellular or cholestatic forms of liver injury, these liver enzymes are released into the bloodstream and, therefore, increased serum levels are diagnostically useful [19]. Therefore, the so-called liver functionality tests measure the serum level of liver enzymes and thus reflect hepatocyte integrity or cholestasis rather than the liver function itself [3]. Thus, in horses with liver disease, serum enzyme levels are related to the concentration of the enzyme in the hepatocyte, the severity, and duration of the disease, and the half-life of the enzyme. In general, the duration of elevation of serum liver enzyme activity depends on molecular size, intracellular location, plasma elimination rate, enzyme inactivation, and increased hepatic production. [19].

2. Types and Characteristics of Hepatic Enzymes in Horses

Standard biochemical indices of hepatocellular disease include sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GLDH), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) while indicators of hepatobiliary disease include γ-glutamyl transferase (GGT). Alkaline phosphatase (ALP) indicates both hepatocellular and biliary origin [3][20]. These enzymes can usually be found in the cytoplasm (AST and LDH), mitochondria (GLDH and AST), nucleus or membranes (ALP and GGT) of hepatocyte cells, where they catalyze specific reactions [20] (**Table 1**).

Table 1. Characteristics of hepatic enzymes in horses based on location and function.

Origin	Type	Location	Function (Catalysis)
Hepatocellular	SDH	Cytoplasmatic	Conversion of fructose to sorbitol
	GLDH	Mitochondrial, in the centrilobular areas of the liver	Conversion of glutamate to 2-oxoglutarate
	AST	Cytoplasmatic of hepatocytes and other tissues, including skeletal muscle	Conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate
	LDH-5	Cytoplasmatic	Reversible transformation of pyruvate to lactate
Biliary	GGT	Microsomal membranes in the biliary epithelium, and also in the canalicular surfaces of hepatocytes <5% is found in the cytoplasm	Cleaves C-terminal glutamyl groups from amino acids and transfers them to another peptide or amino acid. GGT is important in glutathione metabolism (reduced and oxidized GSH are the main targets) and amino acid absorption (cysteine in the kidney)
	ALP	Epithelium of biliary canalicular membrane, and sinusoidal membrane of hepatocytes	Non-specific metalloenzyme which hydrolyzes many types of phosphate esters at an alkaline pH in the presence of zinc and magnesium ion

SDH: sorbitol dehydrogenase; GLDH: glutamate dehydrogenase, AST: aspartate aminotransferase; lactate dehydrogenase (LDH); γ-glutamyl transferase (GGT); alkaline phosphatase (ALP).

Cytoplasmic enzymes are released at the beginning of cell degeneration, whereas mitochondrial enzymes are released after advanced cell necrosis [20]. Based on this, GLDH, AST, and LDH activity are increased in hepatocellular damage while GGT and ALP enzymes are increased in cholestatic liver disease in horses [4][21]. Based on their specificity, liver-specific enzymes include SDH, GLDH, and GGT, where, on the one hand, SDH and GLDH reflect hepatocellular damage and GGT, on the other hand, is indicative of biliary damage. Other enzymes such as AST and LDH also reflect hepatocellular disease while ALP indicates biliary damage. However, these last three enzymes are not specific to the equine liver [2][3] (**Table 2**).

Table 2. Reference value interval, half-life, sensitivity, specificity, and stability of the hepatobiliary enzymes in horses.

Enzyme	Reference Value Interval (UI/l)	Half-Life	Sensitivity	Specificity	Stability
SDH	2–8	<12 h	+++	++++	+
GLDH	2–10 In foals, GLDH increase is compared to adult	12–24 h	+++	+++	++
AST	150–300	7–8 days	+++	+	++++
GGT	5–20	3 days	++++	+++	++++
ALP	120–250 Foal and growing: 100-fold greater than in adults	3 days	++	+	++++

SDH: sorbitol dehydrogenase; GLDH: glutamate dehydrogenase, AST: aspartate aminotransferase; γ-glutamyl transferase (GGT); alkaline phosphatase (ALP) lowest (+); highest (++++).

These parameters are estimated values based on a review of available reports [2][3][20][22][23].

SDH is a liver-specific enzyme in horses. The reference range of SDH in equines is 0–8 IU/L [24]. A drawback is the instability of this enzyme in serum or plasma, even when refrigerated or frozen. According to Fouche et al. [25], plasma

SDH activity in refrigerated samples at 4 °C is suitable for analysis for 24 h. It is not recommended to store plasma for more than 4 h at room temperature or −20 °C. This enzyme has a half-life of 12 h, and after an acute event, baseline values can be observed 3–5 days later [8].

The GLDH enzyme is found in the mitochondria of hepatocytes, and horses with an acute hepatocellular disease and its blood levels are abnormally high. The range of reference plasma values for GLDH in the equine species is <3.5 IU/L [24]. GLDH is more stable compared to other enzymes and has a somewhat longer half-life than SDH and, because of its stability, is a recommended test for detecting acute hepatocellular disease [2][3][23]. However, this parameter should be interpreted with caution in foals, as GLDH levels are usually increased in young lactating foals without actual liver disease [19].

AST is present in the cytoplasm of liver cell mitochondria. The reference range of AST in equines is 150–270 IU/L [24]. Serum levels are usually increased in liver disease and reflect hepatocellular injury. However, since it is also found in the liver, heart, skeletal muscle, and kidney, elevated serum activity can only be interpreted in conjunction with other more specific liver enzymes to diagnose disease. It is also important to note that this enzyme can be increased by hemolysis, as it is present in erythrocytes, and by lipemia [5].

GGT is an enzyme widely distributed in various tissues. Other organs such as the lungs, kidneys, pancreas, and mammary glands also produce GGT. However, these amounts are small, so this enzyme is considered liver specific. However, the activity of this enzyme in serum or plasma originates almost exclusively from hepatocytes [19]. This fact confers to GGT a high specificity for diseases of the hepatobiliary system and its reference range in equines is 5–20 IU/L [24]. Increased GGT activity can be considered normal in foals, donkeys, and mules. In them, serum levels can increase up to 3 times the normal reference range for horses. In foals, during the first month of life, values were 1.5 to 3 times higher than the upper physiological reference values for healthy adult horses. In neonatal foals, serum levels increase during the first two weeks of life because GGT concentrations are higher in colostrum and milk [26].

ALP reflects biliary injury, but is not specific to the liver, as it is also produced in bone, intestine, and macrophages. Care must be taken with the interpretation of this enzyme in growing animals, where normal values are 2 to 3 times higher than reference values in adults due to increased bone turnover associated with physical growth [24]. The reference range of ALP in equines is 73–194 IU/L [24]. In equids, ALP activity is used as a test of liver excretory function. ALP increases after 48 h of liver damage and is usually higher in cholestasis than in hepatocellular damage [24].

LDH isoenzyme 5 (LDH-5) is a non-specific enzyme as it is abundant in the liver, although it is also present in kidneys, muscle, myocardium, and red blood cells [5]. The reference range of LDH in equines is 162–412 IU/L [24].

Based on the clinical point of view of the course of liver or biliary disease, AST and ALP are indicative of chronic disease, whereas SDH, GGT, and GLDH indicate an acute course [3]. The initial activity of an enzyme in plasma or serum is usually a reflection of the amount and turnover of the tissue containing this enzyme. Thus, serum concentrations of specific liver enzymes are usually higher in acute liver disease than in chronic liver disease. Increased activities of SDH, GLDH, and AST occur even with mild acute hepatocellular injury, and the magnitude of the enzyme increase may not correspond to the functional state of the liver [3]. However, in chronic liver diseases with severe fibrosis and a reduction in the number of functional hepatocytes, plasma/serum liver enzyme activities may be within normal limits [4][21].

3. Assessment of Liver Enzyme Abnormalities

The increase in one or more enzymes is usually expressed as increments of levels above the upper limit of the reference interval. Usually, a 2- to 3-fold increase above the reference range is considered mild, while a 4- to 5-fold increase is moderate, and when the value approaches or exceeds a 10-fold increase, it is considered marked (**Table 3**). The degree of the increase in hepatocellular-damage, enzyme activities may help stage disease severity [27].

Table 3. Assessment of liver enzyme abnormalities.

Increase	Number of Times Higher than the Upper Reference Interval
Mild	**<5 times**
Moderate	**5–10 times**
Marked	**>10 times**

Relative increases in hepatocellular versus biliary enzyme activities may point the clinician to a particular process (**Table 4**). Thus, if GGT activity is greatly increased and GLDH, SDH, or AST activity is only moderately increased, a process involving the biliary system, such as cholangiohepatitis, is most likely to be suspected. Conversely, if hepatocyte enzyme activity is very high and GGT activity is slightly increased, then the disease predominantly involves hepatocytes, such as hepatitis [19][20]. Other causes of liver disease may result in a similar increase in hepatocellular and biliary enzyme activities, such as pyrrolizidine alkaloid toxicity and hepatic lipidosis. These processes are probably highly dependent on the duration of the disease. Thus, if serum enzyme activity decreases by 50% for 2–4 days, it suggests that damage has ceased. Conversely, if the activity remains constant over time or increases, it suggests that damage is continuing.

Table 4. Patters of alterations of hepatic enzymes.

Number of Times Higher than the Upper Reference Interval	Pattern
>10-fold increase in SDH, GLDH, and/or AST, and <3-fold in GGT and/or ALP	Hepatocellular predominance (acute necrosis, ischemic or toxic damage to hepatocyte)
>5–10-fold increase in GGT and/or ALP, and <3-fold in SDH, GLDH and/or AST	Biliary predominance (cholestasis, cholangitis, choletithus)
>10-fold increase in SDH, GLDH and/or AST and GGT and or ALP	Mixed liver injury (acute hepatocellular and biliary damage)

References

1. Bergero, D.; Nery, J. Hepatic diseases in horses. J. Anim. Physiol. Anim. Nutr. 2008, 92, 345–355.
2. West, H.J. Clinical and pathological studies in horses with hepatic disease. Equine Vet. J. 1996, 28, 146–156.
3. Divers, T.J. The equine liver in health and disease. In Proceedings of the 61st Annual Convention of American Association of Equine Practitioners, Las Vegas, NV, USA, 5–9 December 2015; Volume 6, pp. 66–103.
4. Durham, A.E.; Newton, J.R.; Smith, K.C.; Hillyer, M.H.; Millyer, L.L.; Smith, M.R.; Marr, C.M. 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.
5. Durham, A. Liver disease. In Equine Clinical Medicine, Surgery and Reproduction, 2nd ed.; Munroe, G., Ed.; CRC Press: Boca Raton, FL, USA, 2020; pp. 875–901.
6. Divers, T.J. Liver Failure and Hemolytic Anemia. In Equine Emergencies Treatment and Procedures, 3rd ed.; Saunders: St. Louis, MO, USA, 2008; pp. 237–258.
7. Gather, T.; Walter, S.; Pfaender, S.; Todt, D.; Feige, K.; Steinmann, E.; Cavalleri, J.M.V. Acute and chronic infections with nonprimate hepacivirus in young horses. Vet. Res. 2016, 47, 97.
8. Divers, T.J.; Barton, M.H. Disorders of the Liver. In Equine Internal Medicine, 4th ed.; Reed, S.M., Bayly, W.M., Sellon, D.C., Eds.; WB Saunders: Philadelphia, PA, USA, 2018; pp. 843–881.
9. Moore, M.E.; Knottenbelt, D.; Matthews, J.B.; Beynon, R.J.; Whitfield, P.D. Biomarkers for ragwort poisoning in horses: Identification of protein targets. BMC Vet. Res. 2008, 4, 30.
10. Bandarra, P.M.; Pavinini, S.P.; Raymundo, D.L.; Correa, A.M.; Pedroso, P.M.; Driemeier, D. Trema micrantha toxicity in horses in Brazil. Equine Vet. J. 2010, 42, 456–459.
11. Caloni, F.; Cortinovis, C. Toxicological effects of aflatoxins in horses. Vet. J. 2011, 188, 270–273.
12. Oliveira-Filho, J.P.; Cagnin, D.Q.; Badial, P.R.; Pessoa, M.A.; Del Piero, F.; Borges, A.S. Hepatoencephalopathy syndrome due to Cassia occidentalis (leguminosae Caesalpinioideae) seed ingestion in horses. Equine Vet. J. 2013, 45, 240–244.
13. Ramsay, J.D.; Evanoff, R.; Wilkinson, T.E., Jr.; Divers, T.J.; Knowles, D.P.; Mealey, R.H. Experimental transmission of equine hepacivirus in horses as a model for hepatitis C virus. Hepatology 2015, 61, 1533–1546.
14. Figueiredo, A.S.; de Moraes, M.V.D.S.; Soares, C.C.; Chalhoub, F.L.L.; de Filippis, A.M.B.; Dos Santos, D.R.L.; de Almeida, F.Q.; Godoi, T.L.O.S.; de Souza, A.M.; Burdman, T.R.; et al. First description of Theiler's disease-associated virus infection and epidemiological investigation of equine pegivirus and equine hepacivirus coinfection in Brazil. Transbound. Emerg. Dis. 2019, 66, 1737–1751.
15. Ramsauer, A.S.; Badenhorst, M.; Cavalleri, J.V. Equine parvovirus hepatitis. Equine Vet. J. 2021, 53, 886–894.

16. Tomlinson, J.E.; Tennant, B.C.; Struzyna, A.; Mrad, D.; Browne, N.; Whelchel, D.; Johnson, P.J.; Jamieson, C.; Löhr, C.V.; Bildfell, R.; et al. Viral testing of 10 cases of Theiler's disease and 37 in-contact horses in the absence of equine biologic product administration: A prospective study (2014–2018). J. Vet. Intern. Med. 2019, 33, 258–265.
17. Tomlinson, J.E.; Jager, M.; Struzyna, A.; Laverack, M.; Fortier, L.A.; Dubovi, E.; Foil, L.D.; Burbelo, P.D.; Divers, T.J.; Van de Walle, G.R. Tropism, pathology, and transmission of equine parvovirus-hepatitis. Emerg. Microbes Infect. 2020, 20, 651–663.
18. Beeler-Marfisi, J.; Arroyo, L.; Caswell, J.L.; Delay, J.; Bienzle, D. Equine primary liver tumors: A case series and review of the literature. J. Vet. Diagn. Investig. 2010, 22, 174–183.
19. Hoffmann, W.E.; Solter, P.F. Diagnostic enzymology of domestic animals. In Clinical Biochemistry of Domestic Animals, 6th ed.; Kaneko, J.J., Harvey, J.W., Bruss, M.L., Eds.; Elsevier: San Diego, CA, USA, 2008; pp. 351–378.
20. Durham, A. Management of horses with hepatic disorders. In The Equine Hospital Manual; Corley, K., Stephen, J., Eds.; Blackwell: Oxford, UK, 2008; pp. 524–532.
21. Satué, K.; Gardon, J.C.; Muñoz, A. Use of Laboratory Testing to Diagnose Liver and Biliary Dysfunction in the Horse. J. Gastroenterol. Hepatol. Res. 2013, 2, 807–813.
22. DeNotta, S.L.; Divers, T.J. Clinical Pathology in the Adult Sick Horse: The Gastrointestinal System and Liver. Vet. Clin. N. Am. Equine Pract. 2020, 36, 105–120.
23. McGorum, B.C.; Murphy, D.; Love, S. Clinicopathological features of equine primary hepatic disease: A review of 50 cases. Vet. Rec. 1999, 145, 134–139.
24. Pearson, E.G. Liver disease in the mature horse. Equine Vet. Educ. 1999, 11, 87–96.
25. Fouche, S.; Oesch, V.; Gerber, H.; Richter, J.; Howard Peters, L.M. Pre-analytical stability of sorbitol dehydrogenase in equine heparinized plasma. Vet. J. 2021, 274, 105706.
26. Gardner, R.B.; Nydam, D.V.; Mohammed, H.O. Serum gamma glutamyl transferase activity in horses with right or left displacements of the large colon. J. Vet. Intern. Med. 2005, 19, 761–764.
27. Webster, C.R.L.; Cooper, J.C. Diagnostic approach to hepatobiliary disease. In Kirk's Current Veterinary Therapy, 15th ed.; Bonagura, J., Twedt, D., Eds.; Elsevier: St. Louis, MO, USA, 2014; pp. 569–575.