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Monthly changes of standard serum liver parameters of clinically healthy horses over a period of 24 months

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Summary: Horses that are in good health sometimes reveal in routine blood screenings increased serum activities of gamma-glutamyl-transferase (GGT), glutamate-dehydrogenase (GLDH) as well as aspartate-aminotransferase (AST) without any corresponding clinical signs. For that reason, the aim of this study was to assess physiological changes of standard serum liver parameters in a population of normal warmblood horses in training over a period of 24 months. The study was conducted between March 2012 and February 2014. The study population consisted of 14 clinically healthy warmblood horses that were used as police horses. Body weight, body condition score and nutrition history were assessed monthly. The nutrient supply was calculated by ration formulation. Throughout the study feed samples of hay, straw, oats and complementary feeds were obtained on a monthly basis as well as blood samples (n = 334). A complete blood count was performed. The clinical chemistry panel consisted of serum hepatic enzyme activity evaluation including GGT, GLDH, AST as well as the analysis of total bilirubin, bile acids, total protein and albumin. The median hematological parameter as the median serum concentrations of bilirubin, bile acids, total protein and albumin were within the upper reference ranges. In certain months the median activities of AST, GGT and GLDH exceeded the upper reference limits. Furthermore, significant seasonal fluctuations ($p < 0.05$) of bile acids, AST, GGT and GLDH were assessed.

Keywords: horse, serum liver enzymes, bile acids, clinical chemistry

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Introduction

Like preventive medical checkups in humans, some horse owners ask for a yearly routine blood screening of their horses in order to obtain information on health and nutrition status. Although several horses are in good health these screenings reveal increased serum activities of gamma-glutamyl-transferase (GGT), glutamate-dehydrogenase (GLDH) as well as aspartate-aminotransferase (AST) without any corresponding clinical signs. Aside from routine medical chekkups, the serum activities of GGT, GLDH and the concentrations of bile acids, conjugated bilirubin as well as of ammonia are assessed with the intention of investigating hepatic damage or disease (*Reed* et al. 2010). Liver disease may be based on toxic, viral, bacterial, parasitic, metabolic, genetic or neoplastic causes (*Reed* et al. 2010, *Schusser* et al. 2007). Also cholelithiasis and serum associated hepatitis have been described (*Reed* et al. 2010). A retrospective case review based on results of hepatic histologic examinations suggested that because of the distribution of lesions within the hepatic lobule, horses appeared to be prone to injury from food and digestive system borne insults, as well as toxins within the systemic circulation (*Hackett* et al. 2016). However, there are also factors other than liver disease that can influence the prior mentioned increase of serum enzyme activities. *Mills* et al. (1998) described a reduction of the serum activities of GGT, AST as well as of GLDH due to an acute inflammatory response. An increase of the serum activity of AST and GGT was induced by increased training intensity of thoroughbreds (*Linder* 1991, *Mack* et al. 2014) as well as due to colic, most severe in association with a right dorsal displacement of the colon (*Gardner* et al. 2005, *Underwood* et al. 2010). In dogs and rats an increase of serum GGT activity was induced by prednisolone treatment and an increase of serum AST, SDH and GLDH by the administration of dexamethasone (*Solter* et al. 1994, *Jackson* et al. 2008). *Çaylı* et al. (2013) showed furthermore that the level of the serum GGT activity is a parameter for subclinical thoracic arteriosclerosis in humans. *Durham* et al. (2003a/b) assessed the prognostic value of various noninvasive tests for liver disease in mature horses in comparison to the results of liver biopsies. According to these studies, it was not possible to distinguish between healthy horses and horses with liver disease with a single serum liver parameter. The serum activities of GGT were significantly increased in horses with liver disease but 86% of the healthy horses also revealed increased GGT values. The level of serum GLDH activity was not correlated to the presence of a hepatopathy and was without any prognostic value (*Durham* et al. 2003a/b). Activation of liver metabolism due to various causes might be responsible for an increase of serum liver enzymes in clinically healthy horses (*Gehlen* et al. 2010).

According to the data of three different veterinary laboratories (Biocontrol, Ingelheim; IDEXX Vet Med Labor, Ludwigsburg; LABOKLIN Bad Kissingen) the reference values for serum liver enzymes differ slightly. The reference values of serum GGT vary between < 22 U/L and < 46 U/L, of serum GLDH between < 8 U/Land < 12 U/L and the values of serum AST vary between < 250 U/L and < 600 U/L. A recent study examined 2014 blood samples of 649 healthy horses of different ages and breeds. The reference values for warmbloods irrespective

of the age for serum GGT were 6.39–44.8 U/L, for serum GLDH 1.39–11.4 U/L. The values for serum AST were 213–627 U/L for warmbloods of an age of 3–17 years (*Köller* et al. 2014). The aim of this study was to assess physiological changes of standard serum liver parameters in a population of healthy warmblood horses in training over 24 months.

Material and methods

Horses

This prospective study was conducted between March 2012 and February 2014. Originally the study population consisted of 27 clinically healthy warmblood horses (24 geldings and 3 mares) used as police horses. However, by reasons not related to the study, 14 horses stayed in the stable for the entire duration of the study. These 14 horses were finally included in the statistics. The median age was 10 years (25/75 percentile: 9/12). The initial median weight of the animals was 620 kg (25/75 percentile: 590/648 kg) and the median body condition score (1/9) was 6, 5 /9 (25/75 percentile: 6/7). The horses were kept in individual box stalls with straw bedding. The horses had access to controlled tap water. Horses were subject to a daily workload as is usual for police mounts. The police horses were used regularly for patrols (3 h walk) in parks, at football matches (mainly at the weekends), at demonstrations and at a carneval procession. For all these the horses were transported. At the football matches the horse were ridden over 2 h, than they stayed over 1,5 h in the trailer and were ridden again over 2 h. Beside patrols, the horses were trained about an hour on a daily basis in an arena, exercised using an automatic walker (1h) and were kept on a paddock for a few hours. The horses were dewormed three times a year (April–September–December).

Health status

Based on a clinical examination by a veterinarian the horses were considered clinically healthy. Diseases and performance of the horses were obtained on a monthly basis by means of a questionnaire. As two horses – one because of colic (December 12) and one because of lameness (July 12) – had to be transported to a clinic, two blood samples could not be taken.

Feeding practice

The nutrition history was recorded monthly. The daily amounts of hay, grains and complementary feeds were weighed and recorded. The nutrient supply was estimated based on nutrient tables *Meyer* and *Coenen* (2002) and the labeling of complementary feedstuffs. Ration formulation was used to control energy and nutrient intake. In case of an inadequate nutrient supply the ration was reevaluated and corrected. Throughout the study feed samples such as hay, straw, oats and complementary feeds were obtained on a monthly basis. All feeds were assessed by sensory control.

Blood sampling

As a part of the monthly health control, blood samples were obtained in the morning between 8 and 9 a.m. before feeding the concentrate. The blood was obtained via a venipuncture of the jugular vein using an 18G cannula. The blood was collected in two 10 mL serum tubes (BD-Plymouth, UK) and in a 4 mL EDTA tube (BD-Plymouth, UK). For serum, the blood was clotted for 20 minutes at room temperature and subsequently centrifuged for 5 minutes at 3000×g. The serum was transferred into tubes and frozen at -20 °C until analysis. The EDTA samples were refrigerated until analysis.

Scaling

The body weight was determined bimonthly by tape measurement (*Carroll* and *Huntington* 1988). Body condition score (1–9) was assessed according to the data from *Schramme* (2003).

Blood analysis

Complete blood counts were performed with the ADVIA 120 hematology system from Siemens. The clinical chemistry panel consisted of serum hepatic enzyme activity evaluation including GGT, GLDH, AST as well as the analysis of total bilirubin, bile acids, total protein and albumin. For all analyses an internal quality control in accordance with the guidelines of the German Medical Association (RiliBäk) was performed. Furthermore, bi-annual proficiency testing of all the parameters used in this study was performed. The concentration of globulin was calculated (concentration of total protein minus albumin). The evaluation of the blood results was based on the reference intervals for 3–17 years old warmbloods (*Köller* et al. 2014).

Microbial evaluation of feedstuffs

In the case of inadequate hygiene, bacteriological and mycological examinations were performed.

Table 1 Number of police patrols at football matches per month

	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
2012			3	4		0	0	1	2	1	3	1
2013	1	1	2	2	2	0	0	1	2	1	2	2
2014	1	2										

Statistical analysis

Statistical analyses were performed with Graph Pad Prism and Excel. Data were assessed for normality with Graph Pad Prism6 and Excel. One-way ANOVA analysis was performed to detect significant changes of analyzed parameters compared to the median value of the respective individual. A Friedman post-hoc test was employed. Correlation of parameters was calculated with Spearman's regression analysis.

Results

During the study, blood samples of 14 horses were obtained. The evaluation therefore included the results of 334 blood samples. According to the questionnaire the performance of the horses was without concern during the whole study.

Feeding

The horses were fed three times a day with hay, complementary feeds and oats. At the beginning of the study the daily ration contained 10 kg hay, 3.1 kg of a complementary feed, 1.8 kg oats, and 100 g of a commercial vitamin and mineral supplement. Furthermore all horses were fed with 290 g cooked linseed in February and March 2013 and from September to October 2013. Except for the vitamin E and sodium intake, the intake of crude protein, energy, calcium, phosphorus, magnesium, iron, manganese, zinc, copper and selenium matched or exceeded the recommendations of GfE (2014).To avoid obesity, at the second month of the study the concentrate intake was reduced by 50% and the commercial mineral and vitamin supplement was exchanged by an enriched Vitamin E containing supplement mixed with minerals and other vitamins. Four horses received water soaked hay.

Workload

The police horses were used nearly 3 days a week for patrols (3 h walk) in parks, at football matches (mainly at the weekends, Table 1), at demonstrations and at a carnival procession. For all these assignments the horses were transported. During the football match duties the horse were ridden over 2 h, than they stayed over 1,5 h in the trailer and were ridden again over 2 h. Beside patrols, the horses were trained about an hour on a daily basis in an arena, exercised using an automatic walker (1h) and were kept on a paddock for a few hours. The horses were dewormed three times a year (April – September – December).

Hygienic quality of feed samples

Bacteriological and mycological examinations for the occurrence of bacteria or fungi were performed in case of sensorial registered changes of quality.

Table 2 Results of microbiological examinations of hay, straw or oats in event of inadequate hygienic quality (findings scoring to schedule of VDLUFA, 2012)

	hay		hay		straw	
2012	Oct	Quality	Nov	Quality	Mar	Quality
Aerobic mesophilic bacteria CFU/g	$2,4 \times 10^7$	Moderate microbial changes	$5,5 \times 10^7$	Moderate microbial changes	$9,5 \times 10^7$	Moderate microbial changes
Bacterium typhi flavum CFU/g	$1,3 \times 10^7$	Moderate microbial changes	$1,3 \times 10^6$	spoilage	$7,0 \times 10^7$	Typical quality
Bacillus spp. CFU/g	$<10^4$	Typical quality	$<10^4$	Typical quality	$2,5 \times 10^7$	spoilage
Staph. spp. (coagulase neg.) CFU/g	$<10^4$	Typical quality	$<10^4$	Typical quality	$3,0 \times 10^6$	Moderate microbial changes
Penicillium spp. CFU/g					$1,0 \times 10^4$	Typical quality

	oats		oats	
2012	Apr	Quality	May	Quality
Aerobic mesophilic bacteria CFU/g	$3,1 \times 10^7$	Moderate microbial changes	$4,2 \times 10^7$	Typical quality
Bacterium typhi flavum CFU/g	$9,0 \times 10^6$	Typical quality	$1,2 \times 10^7$	Typical quality
Bacillus spp. CFU/g	$1,0 \times 10^6$	Typical quality	$<10^3$	Typical quality
Staph. spp. (coagulase neg.) CFU/g	$<10^3$	Typical quality	$<10^3$	Typical quality
Penicillium spp. CFU/g	$2,3 \times 10^4$	Typical quality	$8,0 \times 10^4$	Moderate microbial changes

Hematology

Hematology screening did not reveal any significant monthly variations and all values were in the established reference ranges.

Biochemistry

Median serum concentration of bilirubin, bile acids, total protein and albumin were within the reference ranges (Table 4). During the two years of the study the medians of total protein, albumin, bilirubin and bile acids were never above the reference range.

Figure 1 shows seasonal fluctuations of serum bile acids. The highest medians of serum bile acids were in September 2012 and August 2013. A significant ($P<0.05$) decrease in serum bile acids was noticed in October 2012 and December 2012. In most cases the serum bile acids concentrations did not exceed the upper reference limit. However, in September 2012, April 2013, June 2013 and from July to September 2013 individual horses occasionally showed serum bile acids concentrations in the reference limit. Seven of the 14 horses had serum bile acids levels above the reference values during the entire duration with at least one horse showing these higher serum bile acids concentrations at least in one month. Three horses had exceeded serum bile acids values in two months and four horses in one month. The highest serum bile acids concentration was 25.1 µmol/L.

Serum liver enzyme activities

The median of serum AST (U/L) was the entire study within the reference values (Figure 2). In November 2012 ($P<0.05$) and March 2013 ($P<0.01$) there was a significant decrease in serum AST and in August 2013 a significant ($P<0.05$) increase in serum AST. The highest medians of serum AST were in September 2012 and August 2013. Even if in none month the median activity of serum AST was above the reference limits, in certain months (13/24) individual horses (6/14) had increased serum AST values. Of these horses two had serum AST activities in the upper range in two months. At least one horse showed increased serum AST levels over 3, 5, 6, or 7 months respectively. In the first year of study the relevant months were March (n = 1 horse), April (n = 2), May (n = 2), July (n = 1), August (n = 1), September (n = 5), in the

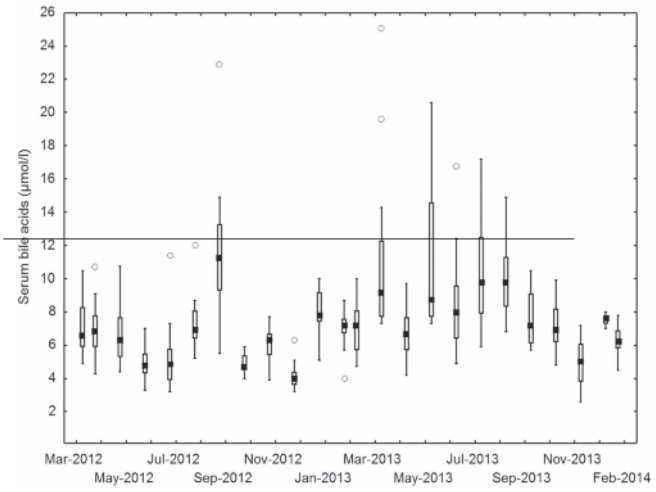

Fig 1 Serum concentrations of bile acids in 14 warmbloods (median, 25 /75 percentile; o potential outlier; * extreme outlier), —— upper reference limit 15 mol/L (*Reed* et al. 2010)

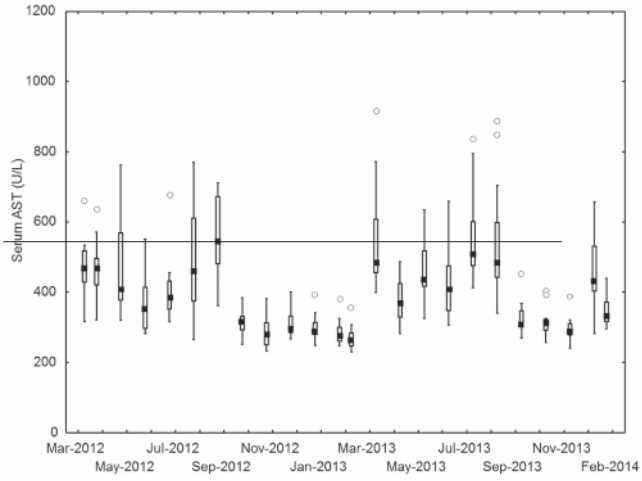

Fig 2 Serum AST U/L from May 2012 to February 2014 of 14 horses (median, 25 /75 percentile; o potential outlier; * extreme outlier) —— reference value 213.2-626.7 U/L (*Köller* et al. 2014)

Table 3 Hematological parameter over 24 months in 14 horses (expressed in median, 25 percentile, 75 percentile, G-10 ^{9}; T-10 ^{6}, reference value *Köller* et al. 2014)

	Median	25 percentile	75 percentile	Reference range
Leucocytes G/L	6	5.3	6.7	4.4 – 12.0
Neutrophiles G/L	3.94	3.29	4.5	2.0 – 6.9
Lymphocytes G/L	1.68	1.5	1.9	1.5 – 5.6
Monocytes G/L	0.22	0.20	0.26	0.2 – 0.6
Eosinophiles G/L	0.08	0.06	0.13	< 0.7
Erythrocytes T/L	7.7	7.5	8.0	6.6 – 9.8
Hemoglobin mmol/L	13.3	12.8	14.0	7.2 – 14.0
Hematocrit L/L	0.4	0.38	0.42	0.27 – 0.4

second year the months April (n = 3), June (n = 1), July (n = 1), August (n = 3), September (n = 3) and January (n = 2). The highest serum AST activity recorded in an individual horse was 990U/L.

The median activity of serum GGT (Figure 3) varied over the entire period of study. There was a large variation in the serum GGT activities and it was interesting that in several months (n = 6) median serum GGT activities were higher than the upper reference limit. The median serum GGT activities rose above the reference values in September to October 2012 and in August to September 2013. Only in 5/24 months (December 2012 to March 2013; December 2013) none of the individual horses revealed increased activities of serum GGT. Furthermore 12/14 horses showed at least in one month serum GGT activities in the upper reference limit. Part of the seasonal fluctuations of serum GGT was significant. The median activity of serum GGT significantly increased in September 2012 and 2013 ($P<0.05$) and there was a significant decrease in January 2013–March 2013 ($P<0.05$). The highest medians of serum GGT were in September 2012 (78 U/L) and September 2013 (83,5 U/L). The highest serum GGT activity in an individual horse was 267 U/L.

The median activity of serum GLDH (Figure 4) increased above the reference value in September 2012, April 2013, August 2013 and September 2013. The highest medians of serum GLDH were in September 2012 and August 2013. In 9/24 months (October 2012, December 2012 to March 2013, October 2013 to December 2013 and February 2014) none of the horses had serum GLDH activities above the reference limit. It was not surprising that 13/14 horses had increased serum GLDH values in one month at least. The highest serum GLDH activity of an individual horse was 327 U/L. The median activity of serum GLDH increased significantly in September 2012 ($P<0.05$) and August 2013 ($P<0.05$) and revealed a significant decrease in February 2014 ($P<0.05$).

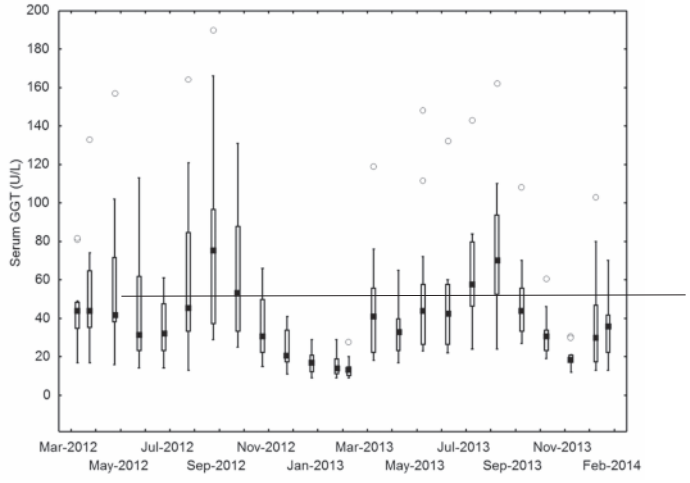

Fig 3 Serum GGT U/L from May 2012 to February 2014 of 14 horse (median, 25 /75 percentile; o potential outlier; * extreme outlier) —— Reference value 45 U/L (*Köller* et al. 2014)

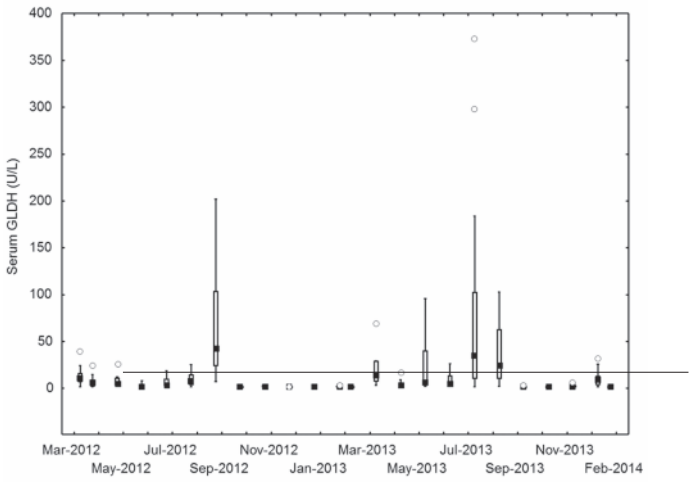

Fig 4 Serum GLDH U/L from May 2012 to February 2014of 14 horses (median, 25 /75 percentile; o potential outlier; *extreme outliner) —— reference values 11.41 U/L (*Köller* et al. 2014)

Table 4 Serum bilirubin, bile acids, total protein and albumin over 24 months in 14 horses (expressed in median, 25 percentile, 75 percentile and reference value *Köller* et al. 2014, *Reed* et al. 2010)

	Median	25 percentile	75 percentile	Reference range
Bilirubin µmol/L	23.9	20.5	27.4	15.07 – 46.96
Bile acids µmol/L	7	5.6	8.3	< 15
Total protein g/L	62	60	65	57 – 78
Albumin g/L	32.5	31.2	34	27.3 – 37
Globulin g/L	29,5	28,8	31,2	24.3 – 44.7

Table 5 Serum AST, GGT and GLDH U/L over 24 months in 14 horses (expressed in median, 25 percentile, 75 percentile and reference value *Köller* et al. 2014)

	Median	25 percentile	75 percentile	Reference range
AST U/L	368	305.5	472	213.2 – 626.7
GGT U/L	22	22	56	6.39 – 44.8
GLDH U/L	3	1.8	10.3	1.39 – 11.41

Correlations

Correlations between weight or BCS and any of the analyzed parameter could not be detected (Table 6).

Discussion

In both years there were significant seasonal variations of serum GGT, GLDH, AST, bile acids and bilirubin. To the authors knowledge a description of these seasonal-based fluctuations of serum liver enzyme activities and bilirubin as well as bile acids has not been published previously. The results of the study showed that the maximum values of serum GGT, AST, GLDH and bile acids were in late summer and/or early autumn. The median serum activities exceeded upper reference limits at this time. During the entire study none of the horses showed any clinical signs of liver disease. Furthermore the serum concentrations of total protein, albumin, globulin, hemoglobin, bilirubin, as well as the values of packed cell volume (PCV), erythrocytes and the leukogram were always within the normal reference ranges. In order to diagnose a hepatopathy certain biochemical parameters of liver function have been reported to be useful (*Gulick* et al. 1980, *Pearson* and Craig 1980, *Engelking* and *Paradis* 1987, *Durando* et al. 1995, *Barton* and *Morris* 1998). However, the retrospective analysis of historical, clinical, ultra sonographic, serum biochemical and hematological data in prognostic evaluation of equine liver disease from *Durham* et al. 2003a,b was unable to find any single test or combination of tests to differentiate the horses with or without a liver disease. Some horses without histopathological variables had activities of serum GGT up to 199U/L and of GLDH up to 44 U/L (*Durham* et al 2003a,b). The present study showed seasonal variations of serum GGT, AST, GLDH and bile acids, which seemed to be similar to the findings published by *Durham* et al. (2003) in healthy horses without liver changes evaluated by histopathological examination in liver biopsies. According to our study, *Gwaze* et al. (2012) and *Baumgartner* and *Pernthaner* (1994) reported a similar seasonal variation of serum AST, CK, GLDH and GGT in goats and sheep. *Gwaze* et al. (2012) suggested that gastrointestinal parasites induced the elevation of GGT in the summer in sheep and goats. The horse population of this study was routinely dewormed three times a year; unfortunately parasitological examinations were not performed. The lack of information about parasites is a limitation of the present study.

Accompanied by the shortening of daylight length an increase of the concentration of adrenocorticotropic hormone (ACTH) is described in the months from August to October (*Copas* and *Durham* 2012, *Beech* 2011, *Donaldson* et al. 2005). These daylight changes correspond well with the fluctuations in serum GLDH, GGT, AST and bile acids in the present study. ACTH stimulates secretion of glucocorticoid steroid hormones from adrenal cortex cells, especially in the zona fasciculata of the adrenal glands. In dogs, the most common serum chemistry abnormality observed in association with hyperadrenocorticism include increased serum alkaline phosphatase activity (ALP), which is found in 85 to 90% of dogs (*Peterson* 1984, *Herrtage* und *Peterson* 1984, *Feldman* und *Nelson* 2004, *Kintzer* und *Peterson* 2006). In horses the pituitary pars intermedia dysfunction is also associated with an increase in serum ACTH concentration and increases in serum liver enzyme activities (*Reed* et al. 2010) or likewise in iatrogenic hyperadrenocorticism (*Cohen* and *Carter* 1992). Further studies are necessary to ascertain whether the seasonal fluctuations in serum ACTH is associated with changes in liver parameters.

Because of sensoric evaluation assessed changes bacteriological and mycological examinations for the occurrence of bacteria or fungi were performed in samples of the year 2012. In October 2012 hay had slightly increased aerobic mesophilic bacteria and bacterium typhi flavum CFU/g. In autumn or late summer 2013 all food samples had an adequate hygienic quality. An increased contamination of bacteria correlated to primary bacterial hepatitis could not be found; furthermore the horses did not show any corresponding clinical symptoms (*Reed* et al. 2010). Mycotoxins are believed to cause hepatic damage. The three most common mycotoxins which affect liver metabolism are aflatoxin, produced by molds of Aspergillus flavus; Rubratoxin, produced by the mold Penicillium rubrum; and deoxynivalenol(DON), produced by certain Fusarium species, which is associated with increased liver enzyme activities in horses (*Reed* et al. 2010, *Schulz* and *Vervuert* 2015). As the contamination of Aspergillus, Penicillium or Fusarium was low; the increased serum liver enzyme activities seemed not to be correlated to an ingestion of mycotoxins. Unfortunately, it was not possible to determine mycotoxin contamination of the feed. In all these considerations, it must be taken into account that significant changes were found in both years in serum GGT, GLDH, AST and bile acids and the described variations were therefore probably not because of alterations in the bacteriological and mycological status. It is not clear whether water quality made have caused the changes as water samples were not specifically analyzed though the horses had access to controlled tap water.

Table 6 Correlations (x-y) between the serum liver enzymes GGT, AST, GLDH and bile acid, pearson's correlation coefficient (r), P<0.05

Correlation (n=334)x-y		r	p-value	Significance
GGT - AST	y=25071x+284,33	0.70	0.70	P< 0.01
GGT-GLDH	y=0,6247x-9,9685	0.50	0.48	P< 0.01
GGT-Bile acid	y= 0,0329x + 5,902	0.40	0.40	P< 0.06
AST-GLDH	y= 0,0151x+2,6071	0.50	0.54	P< 0.01
AST-Bile acid	y=2,62+0,01x	0.50	0.54	P< 0.01
GLDH-Bile acid	y= 0,0373x+6,771	0.60	0.59	P< 0.01

In dogs and rats an increase of serum GGT activity was induced by prednisolone treatment and of AST, SDH and GLDH by the administration of dexamethasone (*Solter* et al. 1994, *Jackson* et al. 2008). In horses stress during transport, temporary housing or exercise provokes an increase of circulating cortisol (*Lindner* et al. 2000, *Stull* and *Rodiek* 2000, *Fazio* et al. 2008, *Kédzierski* 2016). So we can assume that the transportations of the police horses, the patrols at football matches or at the carneval provoked an endogen cortisol secretion and therefore changes in the liver enzyme activities. As we did not determine the serum or salivary cortisol concentrations, it cannot be assessed whether an increase of cortisol concentration may have induced the variations of GGT or GLDH. However, the horses were not transported any more in late summer or early autumn (Tab. 1) than in other months and the most stressful patrol at carneval time was late winter.

In humans markers of liver function such as serum GGT and alanine amino-transferase (ALT) may predict type 2 diabetes. In addition, in healthy individuals, increased serum GGT and ALT were inversely related to insulin sensitivity and significantly associated with higher insulin secretion rates. Both situations reflect a reduced endogenous clearance of insulin and hepatic insulin extraction during an oral glucose tolerance test (*Bonnet* et al. 2011, *Wallace* et al. 2007). In women serum GGT is related to measures of central body fat (*Wallace* et al. 2007). The horses of the present study exhibited a median BCS of 6.5 with the highest value at 7.25. As the ideal BCS for jumping horses or eventing horses is described as 5–5.5 (*Garlinghouse* und *Burrill* 1998, *Garlinghouse* et al. 1999) and for dressage horses up to 6/9 (*Schramme* 2003), the horses of this study were classified as "moderately obese". But neither the body weight nor the BCS showed seasonal induced fluctuations. As the values of serum GGT were significantly increased in some months, there is the possibility that changes of the hydrolysable carbohydrates content of the ration, perhaps because of feeding a new batch of hay provoked an increase in insulin and therefore an increase in GGT. As results of nutrient analysis were not available, it is speculative if in late summer/ early autumn a new batch of hay with higher carbohydrate content was fed and induced an increase in liver enzyme activities by an increased excretion of insulin.

Like hepatitis C virus (HCV) in humans, the newly identified equine hepacivirus (NPHV) displays a predominating liver tropism that may evolve into chronic infections. *Ramsey* et al. (2015) experimentally transmitted NPHV to 4 young adult Arabian horses and 4 foals. The infection was associated with acute and chronic liver disease as measured by elevations of liver-specific enzymes and/or by histopathology. A frequent occurrence of NPHV was found in Thoroughbreds in northern and western Germany with 453 seropositive horses (61.8%) and 134 horses (18.3%) carrying NPHV RNA (*Reichert* et al. 2017). In France *Pronost* et al. (2017) detected NPHV infections in 6.2% of the horses, but the presence of circulating virus was neither significantly associated with biological disturbances nor with clinical hepatic impairment. Further studies are necessary to ascertain whether changes in liver parameters are associated with NPHV.

One major limitation of the study design was that the course of the serum liver enzyme activities was not followed by ultrasound of the liver or by a liver biopsy. Therefore we suggest conducting a follow-up study to confirm the described seasonal variations in another horse population using different tools to assess liver function. The results of this study showed significant seasonal variations in several serum liver parameters in adult horses especially in late summer and early autumn. During this period the activities of serum GGT and GLDH increased above the reference values. For that reason we suggest an adaptation of the standard values according to season. For that a further study with a greater population is required.

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