EVALUATION OF THE VALUE OF GAMMAGLUTAMYLTRANSPEPTIDASE (GGT) ASSAY IN THE DIAGNOSIS OF GASTRIC ULCERS IN HORSES.

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Summary

Gastric ulcers in horses are a complex and multifactorial condition with poorly understood pathophysiology but often high prevalence. This syndrome, divided into two diseases corresponding to the two stomach mucosae, can affect all horses in a population, according to some studies, with effects ranging from underperformance to colic. Gastroscopy remains the gold standard for diagnosis. Other diagnostic methods have been explored, and previous research has shown an increase in gamma-glutamyltranspeptidase (GGT) activity in ulcerated horses.

This study aimed to experimentally determine a threshold value for GGT activity for diagnostic purposes and to gather prevalence data within the studied population.

Two groups of 30 horses, with and without clinical signs compatible with gastric ulcers, were selected and underwent gastroscopy and blood sampling. Overall, non-glandular, and glandular prevalence rates were 90%, 80%, and 37%, respectively, among asymptomatic horses. ROC curve analysis yielded a threshold value of 21 IU/L for serum GGT activity in diagnosing glandular gastric disease. However, various biases and measurement specificities currently make this value impractical for routine use.

Abstract

Gastric ulcers in horses represent a complex, multifactorial condition, the pathophysiology of which is still poorly understood. However, this syndrome, divided into two diseases corresponding to the two stomach mucosae, can affect all horses in a group in certain studies, with effects ranging from underperformance to colic. Gastroscopy remains the gold standard for diagnosis. Other diagnostic methods have therefore been studied, and previous research has shown increased gamma-glutamyltranspeptidase (GGT) activity in ulcerated horses.

The aim of this study was to experimentally determine a threshold value for GGT activity for diagnostic purposes and to gather prevalence data from the horses studied.

Two groups of 30 horses, with and without clinical signs compatible with gastric ulcers, were selected and underwent gastroscopy and blood sampling. The overall prevalence of gastric ulcers among asymptomatic horses was 90%, with 80% and 37% for non-glandular and glandular cases, respectively. ROC curve analysis indicated that a serum GGT activity cut-off value of 21 IU/L could be useful for diagnosing glandular gastric disease. However, various biases and measurement specificities mean that this value is currently of limited practical use.

Introduction

Gastric ulcers in horses constitute a complex and multifactorial condition, known as Equine Gastric Ulcer Syndrome (EGUS), subdivided into two diseases corresponding to ulcers of the two gastric mucosae (1). This condition is highly prevalent (2–5), causing clinical signs ranging from selective appetite to severe colic, including underperformance. Gastroscopy remains the diagnostic gold standard (1), despite its limitations: fasting requirements, expensive equipment, practitioner training, and cost to the owner. Alternative diagnostic methods have been evaluated but are currently unsuccessful (6,7). However, several studies have demonstrated that ulcerated horses exhibit hematological, biochemical (8–11), or salivary changes (12). Among these analytes, GGT was identified as one of the most accessible (13). Therefore, this study aimed to establish the prevalence of EGUS within the sampled population and to determine a diagnostic threshold value for GGT activity.

Materials and Methods

This experimental study was designed as a case-control study involving 60 horses from the first three squadrons of the French Republican Guard cavalry regiment. These sport horses had comparable activity, housing, and diet. Half showed clinical signs compatible with EGUS, while the other half were asymptomatic. Horses were excluded if they had received systemic treatments (especially anti-inflammatory drugs) within four weeks of the study, exhibited significantly elevated AST or ALP activity, were not of a sport breed, had non-comparable activity, or could not undergo gastroscopy.

Blood was collected in EDTA, heparinized, and serum tubes for each horse, followed by classical gastroscopy (14). Lesions were graded according to European College consensus recommendations (1), and body condition scores were recorded on a scale of 5 (15–17). Data were analyzed using Microsoft Excel for Mac and SAS OnDemand for Academics, along with online tools Epitools and easyROC.

Results

Within the sample, 24 (80%) asymptomatic horses and 23 (79%) symptomatic horses had ulcers of the non-glandular mucosa, irrespective of the grade of the lesion. For the glandular mucosa, 19 (37%) and 15 (48%) horses respectively were affected. These figures rise to 27 (90%) and 25 (86%) respectively when the EGUS as a whole is considered.

In addition, it is important to note that the pylorus of fifteen horses could not be visualised, and two others were only partially visualised - one of which was due to the horse's behaviour.

Within the sample studied, there was no statistical association between the presence of clinical signs and EGUS or any of its components (Tab. 1).

Within the sample, with a few exceptions, there was no statistically significant association between EGUS or any of its components (ESGD or EGGD) and age, unit of assignment, body condition score or GGT activity. However, the significance levels of the statistical tests evaluating the association between the EGGD and the assignment platoon on the one hand and between the EGGD and the GGT activity on the other hand were lower than 0.10: 0.07 and 0.06 respectively. There was, however, a significant association between EGUS and the unit of assignment of the horses (Tab. 2). Finally, there was no statistically significant association between the components of equine gastric ulceration syndrome within the sample ($p = 0.96$).

Table 1 Prevalence of gastric ulcers among the two groups of horses

Disease	Asymptomatic (N = 30)	Symptomatic (N = 29)	Significance Level
Non-glandular gastric disease (ESGD), binary	24 (80% [65; 95])	23 (79% [64; 95])	0.95
Non-glandular gastric disease (ESGD), detailed (%)			
Grade 0	6 (20%)	6 (21%)	0.76
Grade 1	3 (10%)	4 (14%)	
Grade 2	13 (43%)	8 (27%)	
Grade 3	7 (23%)	9 (31%)	
Grade 4	1 (4%)	2 (7%)	
Glandular gastric disease (EGGD)	19 (37% [18; 55])	15 (48% [29; 68])	0.37
Equine Gastric Ulcer Syndrome (EGUS)	27 (90% [73; 98])	25 (86% [68; 96])	0.71

IC95%: 95% confidence interval | EGGD: equine glandular gastric disease | ESGD: equine squamous gastric disease | ESGDbinary: equine squamous gastric disease without taking into account the grades of the disease, i.e. considering sick any individual with a grade greater than or equal to 1 | EGUS: equine gastric ulceration syndrome

a (versus not affected by the disease) n (% [IC95%]) | b as EGSD has several grades, this line gives the total, comparing animals with and without EGSD | c details by grade of disease are given in the next five lines.

Table 2 Analysis of the association between EGUS and different exposures within the sample

Exposure	Healthy (N = 7)	Affected (N = 52)	Significance Level
Age (in years) a	7 (6; 10)	9 (7; 12)	0.22
Unit b			0.03
EC1 c	1 (14%)	24 (46%)	
EC2 d	6 (86%)	16 (31%)	
EC3 d	0 (0%)	12 (23%)	
Location of assignment b g			0.22
BCS b			0.37
2.5	0 (0%)	7 (14%)	
3	3 (43%)	30 (59%)	
3.5	4 (57%)	11 (21%)	
4	0 (0%)	2 (4%)	
5	0 (0%)	1 (2%)	
GGT (IU/L) e	9.7 (4.9)	17.2 (23.3)	0.05 f
PAL (IU/L)	441.1 (151.1)	387.5 (150.1)	0.38
ASAT (IU/L)	238.1 (60.6)	262.8 (81.7)	0.45

The ROC curves showed that GGT activity could only be used to diagnose EGGD. In fact, only the area under the ROC curve for EGGD was significantly different from 0.5 (see Tab. 3), and was therefore of diagnostic interest. The threshold value for GGT activity thus determined was 21 IU/L. The positive and negative predictive values obtained were 100% and 88% respectively.

Table 3 Characteristics of ROC curves and tests using GGT to differentiate healthy and diseased individuals

Disease	AUC	Significance (p) a	Threshold b	Sensitivity (Se)	Specificity (Sp)	PPV	NPV
ESGD	0.57	0.55	10	59%	57%	84%	27%
EGGD	0.90	< 0.01	21	80%	100%	100%	88%
EGUS	0.63	0.19	3	100%	0%	88%	NE

Legend: AUC: area under the curve | p: significance level | Se: sensitivity | Sp: specificity | PPV: positive predictive value | NPV: negative predictive value | EGGD: equine glandular gastric disease | ESGD: equine squamous gastric disease | EGUS: equine gastric ulceration syndrome | NE: not evaluated

a : statistical test evaluating the identity of the area under the curve at 0.5 | b gamma-glutamyltranspeptidase activity, in IU/L

A multivariate linear regression model was then used to search for confounding factors, which identified the body condition score as having played a major confounding role in the associations between EGGD or ESGD and GGT activity. However, its inclusion did not result in a significant association.

Discussion

There was no association within the sample between EGUS or any of its components and GGT activity, contrary to the study by Maliverney (8). For EGGD, the significance level was less than 0.10. It can therefore be assumed that this lack of significance is due to a lack of statistical power.

The value of the area under the ROC curve for EGGD (0.9) suggests that GGT is an accurate indicator (18). In addition, the positive and negative predictive values are very good. However, the fact that the cost of false positives is three times higher than that of false negatives restricts the use of this diagnostic threshold to horses with poor or no performance. To calculate this threshold value, the prevalence of EGGD found here was used, and not that of previous studies (4,19). If this did not change the threshold obtained in this work, it would probably be the case with a larger number of horses studied. The influence of race and gender on GGT activity was not quantified here. However, most studies report differences in activity from one race to another (20-27), with the exception of (28). On the other hand, there does not appear to be any difference between the sexes (20,24,25,29). This threshold found should therefore be used with all the more caution when horses of other breeds or performances are studied.

The biochemical analyses were carried out here using automated reagent pairs used in human medicine, so there was no reference range for the horse species, which makes it impossible to know whether the threshold identified at 21 IU/L is part of a reference range or not. This also meant that horses with a presumed increase in AST or PAL activity could not be excluded, as it was not known whether this was real or relative.

During the gastroscopic examinations, 15 pylori could not be visualised, which constitutes a non-differential classification bias.

Body condition score played a strong confounding role in this study according to the confounding search. On the other hand, age did not play a confounding role here. While some studies have concluded that age does not influence GGT activity (20,24,25), others have shown the opposite

(29,30). If such a study were to be repeated, particular attention would need to be paid to the age of the horses in the different groups.

Feeding was not studied here. However, differences in feeding methods from one assignment unit to another could make this exposure a confounding factor, especially as feeding methods have an influence on GGT activity (31,32). The EGGD has been evaluated according to the recommendations (1). However, this descriptive system has poor reproducibility and repeatability (33). A grade system (14) appears to perform better (34). The use of the latter system might have made it possible to analyse the data collected more finely, according to the severity of the ulcers.

This study is the first to identify a threshold value for a blood analyte to diagnose gastric ulcers in horses, according to the available literature. It partially confirms the preliminary results of Maliverney (8).The study led to the determination of a threshold value for glutamyltranspeptidase activity, allowing strong suspicion of ulceration of the glandular gastric mucosa. Although there is room for improvement, this indicator can facilitate screening to select candidates for gastroscopy. It also confirms the high prevalence of EGUS in sport horses and explores this condition in military horses for the first time. In addition, it provided a scientific basis for the 23rd Veterinary Group to test a dietary change for the horses of the Cavalry Regiment.

The first prospect for improvement would be to repeat the analyses on an analyser with reference intervals for the horse, or to establish these for the automaton used.A second point would be to repeat this study, with a few modifications and particular points of attention:

- A precise record of each horse's diet, in order to study this potential confounding factor;

- A larger sample size, to compensate for a lack of statistical power and to allow the study of certain exposures that were not studied here;

- An exhaustive display of all the stomachs, in order to determine the prevalence of EGGD in the population;

- Concurrent measurement of GGT activity in the gastric contents, in order to assess whether the hypothesis of in situ production of GGT can be confirmed or refuted.

In addition, a study of the kinetics of GGT activity during anti-ulcer treatment would enable a decision to be taken as to the value of this measurement for the diagnosis or even the monitoring of healing of gastric ulcers.Finally, while it is currently only possible to use GGT activity measurement as a screening tool, leading to the need for a gastroscopy, it is possible to envisage in the future that this assay, coupled with others that are currently not widely available in the field, could make it possible to avoid a gastroscopy under certain conditions.

Conclusion

This study confirmed the high prevalence of equine gastric ulceration syndrome in sport horses, with prevalences of 90%, 80% and 37% for EGUS, its non-glandular component and its glandular component respectively.This experimental work made it possible to establish a diagnostic threshold of GGT activity for equine glandular gastric disease, at 21 IU/L, with good intrinsic and extrinsic characteristics for this test. However, this test should be used with caution due to the analytical choices made in this study.The results did not show significance for most of the associations studied, but a significant association between the assignment of horses and the prevalence of gastric ulcers suggests an influence of the environment (urban, peri-urban, rural) and diet. Although several biases were identified, none appeared likely to strengthen the associations studied, apart from a selection bias that was difficult to quantify. Multivariate regression highlighted the potential role of body condition score as a confounding factor.Further studies, involving more horses and including feed, are needed to obtain more precise conclusions on the value of GGT assays in the diagnosis of gastric ulcers in horses.

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