Evaluation of a questionnaire to detect the risk of developing ESGD or EGGD in horses

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Highlights

- Equine gastric ulcer syndrome (EGUS) has high prevalence in horses worldwide.
- We evaluated a questionnaire proposed by other authors to assess ESGD or EGGD risk in horses submitted to gastroscopy
- Some answers to questions in the survey correlated with an increased or decreased risk of ESGD or EGGD.
- Owners were able to identify an increased risk and implement strategies to prevent the occurrence of gastric lesions.

Abstract

Equine gastric ulcer syndrome (EGUS) affects various categories of horses worldwide. This syndrome is now divided into two different diseases, based on the presence of lesions on either the squamous (Equine Squamous Gastric Disease, ESGD) or the glandular (Equine Glandular Gastric Disease, EGGD) mucosa. Diagnosis is based on the evaluation of the presence of gastric lesions with gastroscopic examination. As a gastroscopy can be considered expensive by clients, therapy is started often on the basis of clinical signs only. The aim of this study was to validate a questionnaire to detect the risk of developing ESGD or EGGD. The owners of 418 horses that were submitted to gastroscopic evaluation were asked to answer a questionnaire on risk factors for ESGD and EGGD. Horses were divided into three groups based on the results of the questionnaire and their risk of developing gastric lesions. In our population the survey was not useful to detect the presence and the severity of the lesions detected during gastroscopic examination, however answers to some of the questions did correlate with the development of gastric lesions. The questionnaire could therefore be a useful tool to evaluate the risk of ESGD or EGGD. Having owners periodically complete the survey could also make them more aware of changes in the conditions of the horses that could lead to gastric lesions. This could then help them seek advice from veterinarians on how to manage this potential risk.

Keywords

Introduction

Equine gastric ulcer syndrome (EGUS) is not a singular disease, it is a syndrome made up of a collection of diseases with a worldwide distribution. Currently, two main forms of the disease have been described in adult horses, one related to the squamous mucosa (Equine Squamous Gastric Disease, ESGD) and one to the glandular mucosa (Equine Glandular Gastric Disease, EGGD) (Sykes et al., 2015). Up to 100% of equids can be affected by gastric lesions in specific populations, and risk factors can be different between ESGD and EGGD (Tamzali et al., 2011; Niedźwiedź et al., 2013; Sykes et al., 2015; Bonelli et al., 2016; Sgorbini et al., 2017; Zavoshti and Andrews, 2017; Rendle et al., 2018; Sykes et al., 2019).

Clinical signs are non-specific (poor performance, recurrent colic, inappetence, poor body condition, etc.), and sometimes animals can have gastric lesions without symptoms (Sykes et al., 2015). The pathogenesis of ESGD is well described, while the pathogenesis of EGGD remains unknown (Sykes et al., 2019).

The reference standard for the diagnosis of ESGD and EGGD is gastroscopy, however owners can sometimes see this procedure as expensive and/or invasive for the horses, and endoscopic equipment can be difficult to come by in some areas. For these reasons, therapy is frequently started only on the basis of signs without a definitive diagnosis (Sykes et al., 2015).

A questionnaire by Barakat (2016) takes into account management practices and stressful situations (e.g. number of grain meals per day, time spent eating hay, non-steroidal anti-inflammatory drugs, changes in herd dynamics, etc.), scoring them according to their influence on the development of gastric lesions. The answers to the questions are used to divide the animals into risk classes for the development of gastric lesions. However, the questionnaire has not yet been validated in a clinical setting.

The aim of this study was to test Barakat's questionnaire on a cohort population of 418 horses undergoing gastroscopic examination to assess how accurately it could predict the risk of a horse to develop ESGD or EGGD.

Material and methods

Horses

A population of horses undergoing gastroscopic examination was evaluated as part of a study on the prevalence of gastric lesions in central Italy. Animals that were at least one year old were included in the study, without exclusions in terms of sex, breed, or level of activity.

Survey

Before the gastroscopy, Barakat's questionnaire (table 1) was presented to the owners. An interviewer posed the questions to the owners, recording the answers on a paper version of the questionnaire. Although the version by Barakat (2016) was formulated in English, the questions were asked in Italian because the study was carried out in Italy. Information was based on events occurring in the three months prior to the gastroscopic exam.

The questionnaire was made up of five close-ended questions on management factors. The questions focused on the time spent eating, the number of grain meals per day, treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) and days

spent in intense work. One question covered possible stressful situations (e.g. travelling, changes in stable mates and herd dynamic injuries and illnesses). Question 1, 2, 3 and 5 had only one possible answer out of 3. Each answer was graded according to the decreased probability of developing gastric lesions (5, 2 or 1 points each). Only for question number 1, a score of 0 was also allocated if horses were not fed any grain.

No questions regarding the kind of grain fed were present. Question 4 described five possible stressful scenarios; each scenario scored 1 point and the owner was asked to mark all the scenarios that could apply to their animals (for a maximum of 5 points for answer 4).

The scores for each question were summed and the horses divided into 3 groups, according to the score of their answers and their risk of developing gastric lesions (Barakat, 2016): horses with a total score ranging between 0 and 5 were considered at low risk, between 6 and 15 at medium risk, and 16 or above at high risk. The results were then recorded on an Excel spreadsheet, along with the results of the gastroscopy.

Gastroscopy

Gastroscopy was performed according to the literature (Sykes et al., 2015) by two operators. The examination was carried out using a 300 cm endoscope (60130PKS, Karl Storz Endoskope, Germany) connected to a processor (Tele Pack Vet X LED, Karl Storz Endoskope, Germany). The video of the endoscopy was recorded and reviewed later to grade the lesions by one clinician with 10 years of experience in gastroscopic examination and blinded to the results of the survey. ESGD was graded according to Sykes et al. (2015), considering horses positive for non-glandular lesions if they had at least grade 2/4. Lesions of the glandular mucosa were described qualitatively, because no grading system has been reported in the literature (Sykes et al., 2015; Rendle et al., 2018): presence/absence of lesions was recorded on the spreadsheet for statistical analysis, considering animals positive for EGGD if any alteration of the mucosa was identified (hyperemia, erosions or ulcers).

Statistical analysis

An unsupervised hierarchical clustering analysis (HCA) was used to organize the horses according to the similarity or dissimilarity of the answer to questions 1, 2, 4 and 5. Question 3 was not used i n the clustering analysis, because the same answers were given for all the horses. The HCA was performed using Euclidean distances and the average linkage method. The relationship between horses and the answers was shown as a dendrogram, in which branch length is determined by the correlation between the results of the answers. The scores per question, the total score and risk class were evaluated with the following linear model:

$$y_{ij} = \mu + CLUSTER_i + \varepsilon$$

where

y_{ij} = question score, total score and risk class

$CLUSTER_i$ = fixed effect of the ith cluster effect (Cluster_1, Cluster_2, Cluster_3, Cluster_4

ε_{ij} = casual error.

The chi-square test was used to determine which cluster was related to the occurrence or not of lesions in order to evaluate the influence of a different combination of risk factors in the development of ESGD or EGGD. The chi-square test compares the observed frequencies (number of horses with or without lesions) with those expected. The latter were calculated by multiplying the probability of extracting a horse from a particular cluster and the probability that it has a lesion or not. The expected frequencies indicate the distribution of the subjects between affected and not affected by randomness and not for a specific reason. If the p-value <0.05 the observed frequencies are different from those expected, so it is an indication that the particular stress level defined by the cluster influences the greater or lesser predisposition to contract the ulcer. Finally, the relationship between risk class (Low, Medium and High) and ESGD or EGGD occurrence was estimated by logistic regression analysis, since data were expressed in binary (yes or no) forms. Logistic regression is a statistical model that in its basic form uses a logistic function to model a binary dependent variable, although many more complex extensions exist. Mathematically, a binary logistic model has a dependent variable with two possible values, such as yes/no which is represented by an indicator variable, where the two values are labeled "0" and "1".

Results

A total of 418 horses were included in the study. The horses were aged between 1 and 28 years (median age 10 years). Different breeds and sex were included (graph 1). To reduce the number of breeds, they were grouped as follow: hot-blooded breeds (Thoroughbreds, Arabians, Quarter Horses) (Benkert, 2019); warm-blooded breeds (Saddlebred horses) (https://en.wikipedia.org/wiki/Warmblood; Benkert, 2019); Baroque horses (Friesians, Andalusian and similar (https://en.wikipedia.org/wiki/Baroque_horse; Benkert, 2019); cold-blooded horses (draft horses); Ponies (https://horsesandfoals.com/hot-warm-blooded-vs-cold-blooded-horses/; Benkert, 2019).

Horses with ESGD with grade 0/4 and 1/4 or hyperemia (224/418, 54%) were categorized as normal. ESGD (grade 2-4/4) (Sykes et al., 2015) was diagnosed in 194/418 horses (46%), while EGGD (any kind of alteration of the mucosa) was diagnosed in 61/418 animals (15%). Of the 61 horses affected by EGGD, 56/61 (91.8%) were contextually affected by ESGD, while only EGGD lesions were observed in 5/61 (8.2%) subjects.

The administration of the questionnaire and the record of the answers on a paper version required 5 to 10 minutes on average. Based on the results of the survey, 92% of the horses (386/418) were considered to be at medium risk, 4% (18/418) at high risk, and 3% (14/418) at low risk for gastric lesions. The total score for the questionnaire ranged from 0 to 25 points and the classification of horses based on the score of each question is reported in table 2.

A dendrogram was created which enabled us to group the animals into four clusters, with an 82.80% similarity level. Cluster_2 and Cluster_3 were the most numerous groups (211 and 148 respectively), while 41 and 18 horses were classified in Cluster_1 and Cluster_4, respectively (figure 1).

Cluster_1 was characterized by horses that received two or more grain meals per day, spent more than 50% of the day grazing or eating hay, showed a low level of stress as defined by question_4 and were never involved in intense work, except for an occasional short gallop for fun (table 3). Horses in Cluster_2 showed similar characteristics as those of Cluster_1, except for having only one grain meal per day. Cluster_3 differed from Cluster_2 in terms of a higher stress stimulus level, as defined by question_4. Finally, Cluster_4 represents horses that received one grain meal per day, spent less than 50% of the day grazing or eating hay, and spent three or more days per week in intense/high-speed work (table 3).

There was a significant increase in total scores from Cluster_1 (5.24) to Cluster_4 (16.22). These results are related to a significantly lower level of risk for Cluster_1 and a higher level for Cluster_4, while Cluster_2 and Cluster_3 showed intermediate values (table 3).

The relationship between clusters and lesion occurrence estimated by ESGD or EGGD are reported in tables 4 and 5, respectively. The chi-square test showed that horses in cluster_1 had a significantly lower occurrence of lesions, while horses in cluster_4 had a significant predisposition to the diseases. In contrast, cluster_2 and cluster_3 were not associated with a significant occurrence or non-occurrence of lesions.

The logistic regression analysis demonstrated a significant higher and lower lesion occurrence for high and low risk class, respectively. The effect was observed for both ESGD (table 6) and EGGD (table 7).

Discussion

ESGD and EGGD (Sykes et al., 2015) are worldwide diseases that affect horses of different breeds, ages, sex, and activities. Risk factors for each disease have also been recently described (Vatistas et al., 1999; Lorenzo et al., 2002; Frank et al., 2005; Luthersson et al., 2009; Sykes et al., 2015; Sykes et al., 2019). High prevalence of EGGD has been reported in horses typically considered at relatively low risk of ESGD (Sykes et al., 2019).

The aim of this study was to test Barakat's questionnaire on a cohort population of 418 horses undergoing gastroscopic examination to assess how accurately it could predict the risk of a horse developing ESGD or EGGD. In our population, the prevalence of ESGD is higher respect to EGGD and most of the horses presenting EGGD were also affected by ESGD. This result is in line with literature that reported the presence of ESGD a risk factor to develop EGGD (Sykes et al., 2019).

In our population, nearly all horses were considered at low to moderate risk of developing both ESGD and/or EGGD after analysing the answers given by the owners to the questionnaire and a statistical difference was found between cluster 1 (lower level of risk) and cluster 4 (higher level of risk), but not for the intermediate levels (cluster 2 and 3). Our results could support the hypothesis that the use of the questionnaire could be of benefit for the owners from periodically answering the questions, so that they could identify at risk periods and implement specific countermeasures to prevent the development of gastric lesions. Furthermore, the questionnaire could partially be useful to better select animals to be submitted for gastroscopic examination (i.e. cluster 4 and not cluster 1). Our results showed the limitation of the questionnaire in identifying the intermediate level risk horses in which gastric lesions could be underdiagnosed because not selected for gastroscopy.

By dividing the horses into clusters, it was possible to evaluate the influence of a different combination of risk factors in the development of ESGD or EGGD. In our population, there was no difference in the risk factors identified with the questionnaire for ESGD or EGGD, probably because the questions were designed to detect both ESGD and EGGD (Sykes et al., 2015; Rendle et al., 2018; Sykes et al., 2019). In particular, in our population we found a higher risk of developing ESGD or EGGD in relation to a high level of stress (i.e. intensity, duration or frequency of the exercise, travelling, changes in the environment), and management practices that reduce the amount of time the horse spends eating. In particular, the increment of feeding high grain diets or the exercise intensity are associated to ESGD (Sykes et al., 2019), while the stressful events or the frequency of exercise have been associated to the development of EGGD (Pedersen et al., 2018; Sykes et al., 2019).

Our results showed that the main risk factors determined by this questionnaire are the number of grain meals per day and the intensity of the exercise performed. These risk factors distinguish cluster_1 and cluster_4 statistically, which are those least and most probable of developing ESGD, but not EGGD. The significant effect obtained for cluster_1 and cluster_4 in the χ^2-square test (tables 4 and 5) demonstrates that the distribution, between horses with and without lesions, observed in the respective groups, is different from that which should be expected under conditions of statistical randomness. In practice, this result means that the highest number of horses without lesions in cluster_1 and with lesions in cluster_4 is due to the typical stress state of the respective cluster. In this way, it is highlighted how subjects exposed to greater risk factors lead to greater exposure to contract lesions. Grain meals have been associated with an increased prevalence of ESGD, because of the high levels of volatile fatty acids (VFA) produced during the fermentation of digestible carbohydrates by the bacteria in the stomach. VFAs are responsible for damage to squamous mucosa in an acidic gastric environment (Nadeau et al., 2003a, 2003b; Andrews et al., 2008). The lesions of the stomach lining are worsened by a population of bacteria that are able to ferment carbohydrates, producing lactic acid and thus further lowering the pH of the stomach (Rafat et al., 2008; Al Jassim and Andrews, 2009; Perkins et al., 2012). A big grain meal provides the bacteria with a substrate, leading to large amounts of VFA and lactic acid, increasing the probability of gastric ulcerations, especially in the squamous mucosa (Nadeau et al., 2003a, 2003b; Andrews et al., 2008). In this study, only the number of grain meals has been included in the questionnaire. This could be a limitation in the evaluation of the risk factors because they could be influenced also by the kind of grain (for example, complete vs sweet grain). It could be useful for future studies to improve the questionnaire in the part regarding the grain meal. The relationship between EGGD and diet has not been proven (Rendle et al., 2018), however our data show that feeding horses a grain meal only once a day might be associated with the development of gastric lesions not only in the squamous, but also in the glandular mucosa (also if not associated with ESGD). Further studies including horses affected only by EGGD are needed to verify if the diet (number of meals, type of grain) might be a risk factor for the development of gastric lesion in the glandular stomach.

The intensity and the duration of the exercise are considered one of the main causes of both ESGD, while the frequency of exercise has been reported as a risk factor for EGGD in horses (Sykes et al., 2019). The pathogenetic mechanism in ESGD is the increase in intraabdominal pressure enhances the level of gastric content, exposing more of the squamous mucosa to an acidic environment and causing lesions (Sykes et al., 2015; Sykes et al., 2019). For EGGD, recently a relationship between the frequency of exercise and the gastric blood supply was proposed as a cause of glandular disease (Sykes et al., 2015; Rendle et al., 2018; Sykes et al., 2019). Another

hypothesis proposed is that exercise could be an example of physiological stress on the glandular mucosa (Rendle et al., 2018), as long as the type of trainer or the number of caretakers and riders (Monki et al., 2016).

The other risk factors considered in the questionnaire (changes in management and environment, time spent eating, use of non-steroidal anti-inflammatory drugs, illness or injury) are all considered stressful situations for horses. Management factors have been implicated in the development of gastric ulcers in the squamous mucosa (Sykes et al., 2015; Zavoshti and Andrews, 2017). Intermittent feeding reduces the amount of saliva produced and ingested, decreasing its buffering effect on the acidic environment of the stomach (Luthersson et al., 2009; Videla and Andrews, 2009; Sykes et al., 2015). Changes in the environment could cause stress in horses, which is considered a primary cause of gastric lesion (Sykes et al., 2015). A preliminary study on pleasure horses in Italy found an increased risk of developing both ESGD and EGGD in animals that lived with the owner for less than six months before the gastroscopy (Busechian et al., 2015). Similarly, a recent study identified an increased number of trainers and caretakers as a potential risk factor for EGGD (Sykes et al., 2019). These results could support the hypothesis that changes in the management and environment could be stress factors in the development of gastric lesions, in particular EGGD. In this study we analysed the questionnaire answers not individually, but grouped in clusters; thus, the management and changes in the environment have not been evaluated alone.

The main limitation of the study is the language in which it was proposed: the interviewer, fluent in English, translated the questionnaire from English to Italian before posing the questions to the owners. Not all owners were able to understand English, and translating it made it less difficult for them to misinterpret the survey. Furthermore, the questions were posed by the interviewer, and explanations were given if the meaning was not clear: none of the owners, however, asked for further clarifications. Moreover, a possible inaccurate information could have been provided by the owners. Another limitation of the study was the population of horses investigated, that reflects the animals usually presented in our area for gastroscopic examination: the majority are pleasure horses, with a small number of racehorses' present. Finally, the questionnaire was designed to detect not singularly, but contextually ESGD and EGGD.

Conclusions

The questionnaire is useful as a screening tool for the owners to detect possible at-risk situations in their horses and implement preventive measures to reduce the

incidence of EGUS. Horses that are exercised frequently and at a high intensity and that are fed one grain meal per day, are at increased risk of developing gastric lesion in both mucosae. These situations could also be made worse by concurrent stressful situations, such as changes in management and environment, or concurrent illnesses. Having the owners periodically answer the survey questions could potentially make them aware of the risk situation of their horses and seek the advice of veterinarians for the diagnosis and treatment of gastric ulcers.

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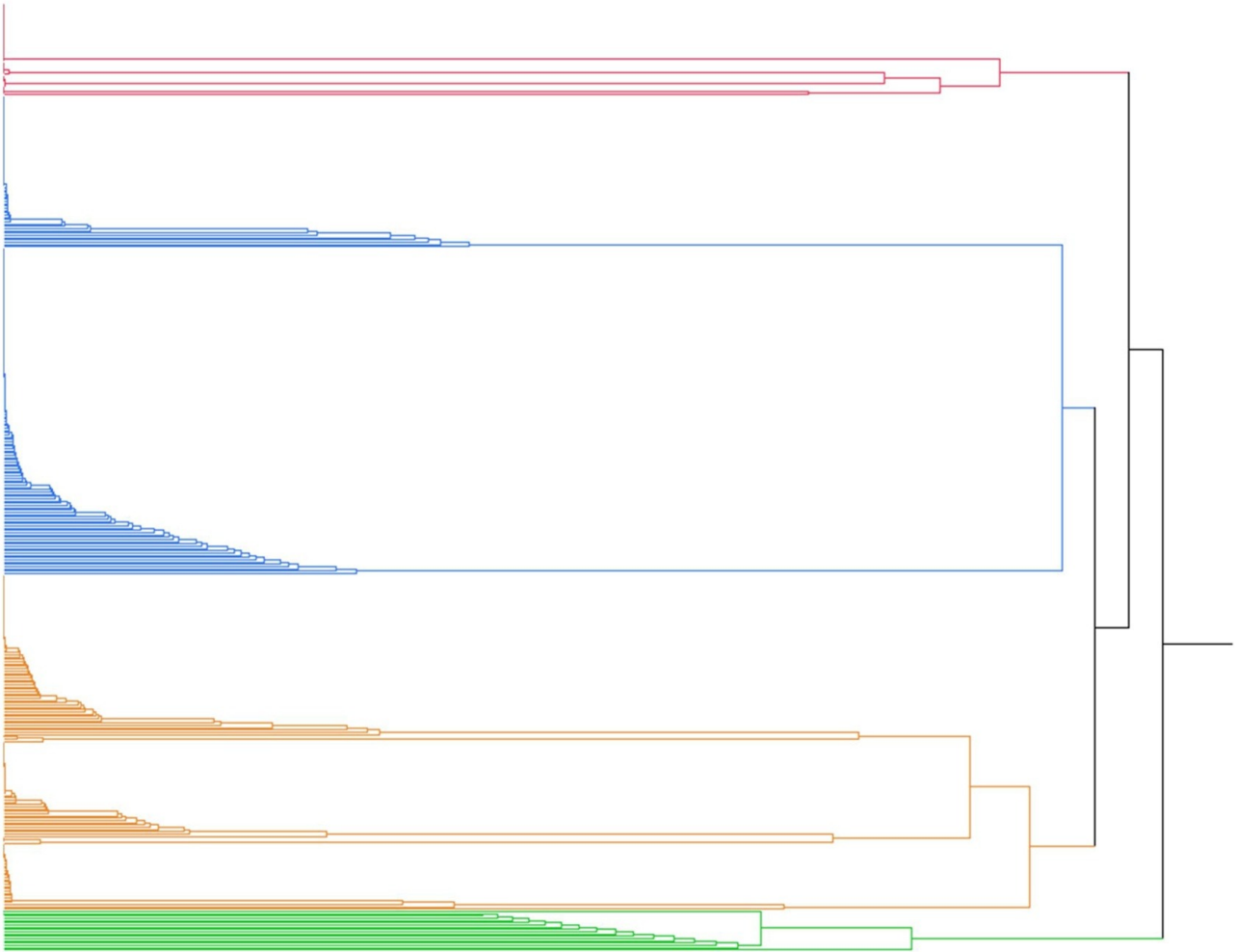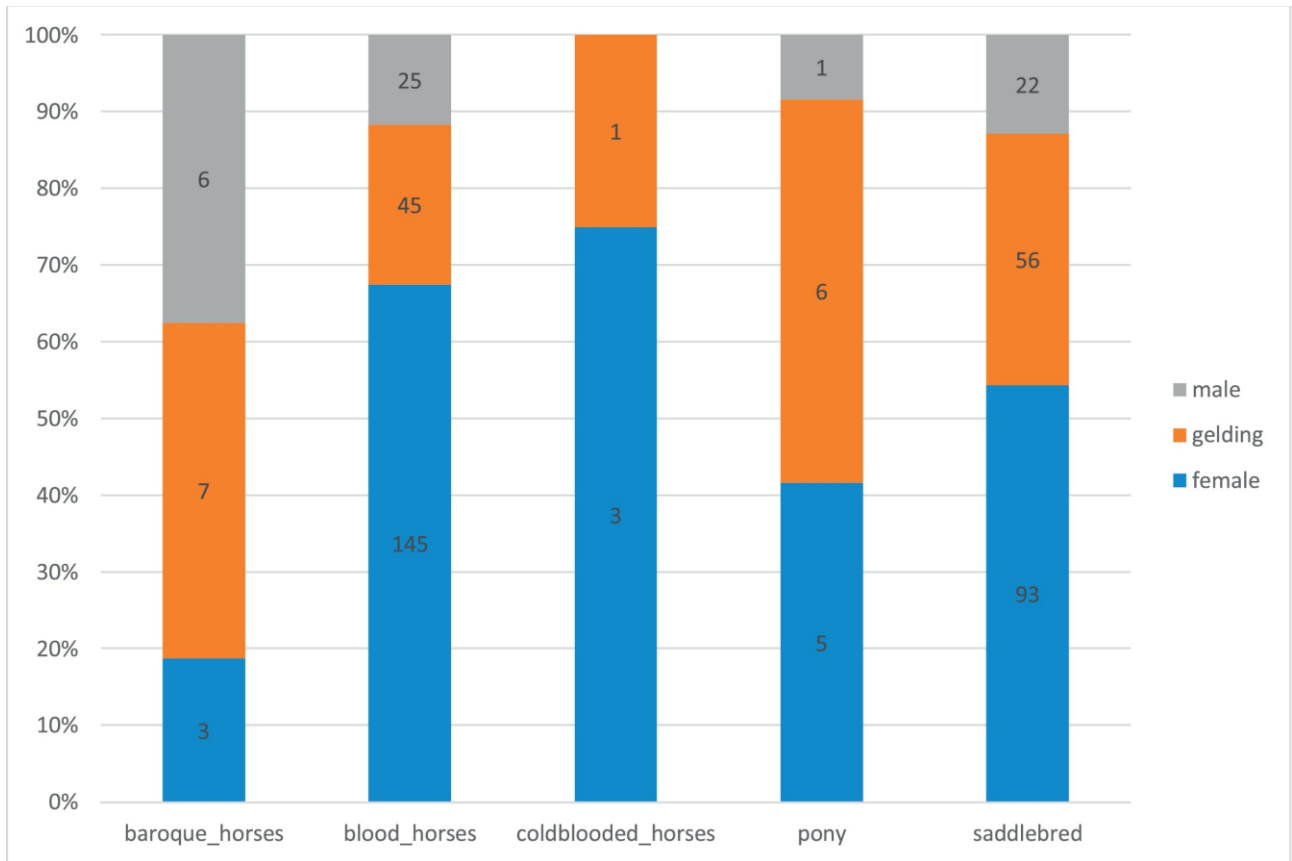
Figure 1: Dendrogram of Hierarchical Clustering Analysis. Red = Cluster_1; Blue = Cluster_2; Orange = Cluster_3; Green = Cluster_4.

Graph 1: Distribution of the population by breed and sex

Table 1: Gastric risk calculator proposed by other (Barakat, 2016)

Gastric ulcer risk	Score		
	1	2	5
How many meals per day is your horse's grain ration?	3 or more	2	1
How much of the day does your horse spend grazing or eating hay?	75%	50%	25%
How many days in a row has your horse been on NSAIDs in the past 3 months?	None	10 or less	More than 10
Over the past 3 months, which of the following apply to your horse?	Spent 1 day away at show, clinic or other?		
	Took a trailer ride		
	Attended competition or clinic at unfamiliar location		
	Change in herd dynamics		
	Sustained injury or Developed illness		
How many days per week is your horse in intense work?	None	1 or 2	3 or more
Total score Risk score: 0-5 low, 6-15 moderate, 16-25 high			

Table 2: Distribution of answers given by the owners for each question

Question 1: How many meals per day is your horse's grain ration?	
No grain (0 points)	15
3 or more (1 point)	0
2 (2 points)	26
1 (5 points)	377
Question 2: How much of the day does your horse spend grazing or eating hay?	
75% (1 point)	105
50% (2 points)	295
25% (5 points)	18
Question 3: How many days in a row has your horse been on NSAIDs in the past 3 months?	
None (1 point)	418
Less than 10 (2 points)	0
More than 10 (5 points)	0
Question 4: Over the past 3 months which of the following apply to your horse? (1 point each)	
Spent 1 day away at show, clinic or other?	48
Took a trailer ride	155
Attended competition or clinic at unfamiliar location	48
Change in herd dynamics	112
Sustained injury or developed illness	7
Question 5: How many days per week is your horse in intense work?	
None (1 point)	400
1 or 2 (2 points)	0
More than 3 (5 points)	18

Table 3: Effect of Cluster group on question scores

	Cluster_1	Cluster_2	Cluster_3	Cluster_4	SEM[1]	P-value[2]
Nº of horses	41	211	148	18		
Question_1	1.27^B	5.00^A	5.00^A	5.00^A	0.05	***
Question_2	1.78^B	1.68^B	1.80^B	5.00^A	0.07	***
Question_4	0.19^B	0.00^B	2.31^A	0.22^B	0.06	***
Question_5	1.00^B	1.00^B	1.00^B	5.00^A	0.05	***
Total score	5.24^D	8.68^C	11.11^B	16.22^A	0.11	***
Risk class	1.66^C	2.00^B	2.00^B	3.00^A	0.02	***

[1]: standard error of the mean [2]: ***= P-value < 0.001; $^{A-D}$= Means within a row with different letters differ significantly (P≤0.001).

Table 4: Contingency table and chi-square test for the estimation of relationship between cluster groups and ulcer occurrence by ESGD

Observed				**Expected**				P-value[1]
	No	Yes	Total		No	Yes	Total	
Cluster_1	38	3	41	Cluster_1	22	19	41	***
Cluster_2	112	99	211	Cluster_2	112	99	211	ns
Cluster_3	73	75	148	Cluster_3	78	70	148	ns
Cluster_4	1	17	18	Cluster_4	10	8	18	***
Total	221	197	418	Total	221	197	418	

[1]: *** = P-value < 0.001, ns = not significant.

Table 5: Contingency table and chi-square test for the estimation of relationship between cluster groups and lesion occurrence by EGGD.

Observed	No	Yes	Total	**Expected**	No	Yes	Total	P-value[1]
Cluster_1	41	0	41	Cluster_1	35	6	41	***
Cluster_2	180	31	211	Cluster_2	181	30	211	ns
Cluster_3	124	24	148	Cluster_3	127	21	148	ns
Cluster_4	13	5	18	Cluster_4	15	3	18	***
Total	221	197	418	Total	221	197	418	

[1]: *** = P-value < 0.001, ns = not significant.

Table 6: Effect of Risk class on the lesion occurrence by ESGD, using logistic regression. Data expressed number of animals (% on the total of each class).

Risk class	Ulcer occurence			Odd ratio	P-value[1]
	No	Yes			
Low	12(86%)	2 (14%)	Low vs Medium	5.00	***
Medium	211 (55%)	175 (45%)	Low vs High	101.91	
High	1 (6%)	17 (94%)	Medium vs High	20.48	

[1]: *** = P-value < 0.001

Table 7: Effect of risk class on the lesion occurrence by EGGD, using logistic regression. Data expressed number of animals (% on the total of each class)

Risk class	Ulcer occurence			Odd ratio	P-value[1]
	No	Yes			
Low	14 (100%)	0 (0%)	Low vs Medium	10376.77	*
Medium	331 (86%)	55 (14%)	Low vs High	4483.01	
High	13 (72%)	5 (28%)	Medium vs High	2.31	

[1]: * = P-value < 0.05