Risk factors for Equine Gastric Glandular Disease: A case-control study in a Finnish referral hospital population

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2016


http://hdl.handle.net/10138/166635
https://doi.org/10.1111/jvim.14370

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Risk Factors for Equine Gastric Glandular Disease: A Case-Control Study in a Finnish Referral Hospital Population

J. Mönki, M. Hewetson, and A.-M.K. Virtala

Background: Equine gastric glandular disease (EGGD) is a term used to classify erosive and ulcerative diseases of the glandular mucosa of the equine stomach. Epidemiologic studies of risk factors for EGGD have not been reported.

Objective: To determine risk factors for EGGD.

Methods: Retrospective case-control study. The data were analyzed by multivariable logistic regression modeling. Analysis was performed on the full dataset. An additional analysis compared horses with glandular lesions (n = 43) against healthy horses (n = 22).

Results: On first analysis, Warmblood breed (OR = 13.9, 95% CI 2.2–90.9, P = .005) and an increasing number of caretakers (OR = 7.3, 95% CI 0.98–55.6, P = .053) were associated with an increased risk of EGGD. On analysis of the subset of data, Warmblood breed (OR = 28.6, 95% CI 2.96–250.0, P = .004) and increasing number of riders (OR = 12.99, 95% CI 0.94–166.7, P = .056) were risk factors. The presence of sand in the colon appeared to have a protective effect against EGGD (OR = 0.195, 95% CI 0.04–1.0, P = .051 for sand versus not having sand).

Conclusions and clinical importance: This study suggests that Warmbloods are predisposed to EGGD and multiple handlers/riders might increase the risk of EGGD. Identification of risk factors allows speculation on potential pathophysiological mechanisms of EGGD.

Key words: Equine gastric ulceration syndrome; Horse; Management; Stress; Ulcer.

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Submitted October 5, 2015; Revised March 23, 2016; Accepted June 11, 2016.

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DOI: 10.1111/jvim.14370

Equine gastric ulcer syndrome (EGUS) has been used for many years as a general all-encompassing term to describe ulcerative diseases of the equine stomach; however, recently it has been suggested that emphasis should be placed on clearly differentiating the affected area of the stomach when communicating research and clinical findings. In lieu of this, the term equine glandular gastric disease (EGGD) has been suggested as a descriptive term to classify a variety of erosive and ulcerative diseases seen in the glandular mucosa of the stomach of the horse that appear to be clinically distinct from equine squamous gastric disease (ESGD). The lesions in the gastric glandular mucosa can be focal or diffuse and can appear as depressed, flat, or raised lesions; with the epithelium being hyperemic, hemorrhagic, fibrinosuppurative, or ulcerated.

Until recently, the prevalence of EGGD has not been reported, primarily because the majority of studies have focused on EGUS in general, without specifically differentiating between horses with squamous and glandular disease. Based on the few studies that are available, the prevalence of EGGD ranges depending on the horse population and discipline: 47–65% in Thoroughbred racehorses; 16–35% in endurance horses; 55–64% in sport horses; and 54–57% in leisure horses and horses used for a variety of purposes.

As the concept of differentiating EGGD and ESGD is rather a new one, there is a paucity of information on risk factors specifically associated with EGGD. Common known risk factors for EGUS (EGGD and ESGD not separated) are intense exercise, a high-grain-low roughage diet, water deprivation, fasting, hospitalization, and overdose of NSAIDs. In the few studies that have considered EGGD as a separate entity from ESGD, the risk factors have been mostly different for these two syndromes. For example, the amount of starch in the diet increased EGUS scores in the squamous region but not in the glandular region. Feeding alfalfa hay was shown to have a protective effect for peptic injury to the gastric squamous mucosa in adult horses whereas weanlings that were fed alfalfa chaff ad libitum had an increased risk of EGGD. In one study, endurance horses had a higher prevalence of EGGD during the competition season when compared to the off-season, whereas in another study increasing amounts of exercise in racing...
Thoroughbreds did not increase the prevalence of EGGD. Furthermore, there was no difference in the mean glandular mucosal lesion scores between Thoroughbred racehorses that had raced compared to horses that had not raced during last 2 months. The effect of exercise on EGGD therefore remains unclear.

Equine glandular gastric disease appears to be an emerging disease, particularly in sport horse populations, and the prevalence and clinical relevance of this disease has recently been highlighted. To date very little is known about the underlying etiopathogenesis of EGGD. Therefore, the objective of this study was to investigate risk factors for EGGD, thus enabling us to speculate on the potential underlying pathophysiological mechanisms of EGGD, and in doing so, to stimulate hypothesis-led investigations into the pathogenesis of this disease.

Materials and Methods

Potential risk factors for EGGD in Finland were investigated by performing a retrospective case-control study. A causal web diagram (Appendix S1) was developed based on biologically plausible hypotheses for EGGD, and was used to identify potential risk factors to be included in a web-based questionnaire that owners were asked to complete. The variables were chosen based on the putative causes of EGGD. Retrospective information was collected from each horse going back 1 year prior to gastroscopy.

Subjects

Any adult horse that underwent gastroscopy in the hospital between August 2013 and December 2014 was a potential case or control, depending on the diagnosis. During the study period, the first author (JM) collected from the hospital records on a weekly basis those horses that had undergone gastroscopy. The medical records of those horses were then reviewed to make sure that the whole stomach was visualized during the procedure and that the findings were recorded appropriately.

The questionnaire administration

The questionnaire was administered as an Internet survey by means of multiple contact modes (phone, SMS, e-mail). The questionnaire was on a University server which provides ready-to-use graphics and secure data transmission.

We approached the owners of suitable horses within 1–2 weeks from the gastroscopy via telephone (a phone call followed by a SMS if the owner did not answer our call) inquiring about their willingness to participate in the study after briefing about the purposes of the study. The link to the questionnaire was sent right after their consent was obtained. If an owner had not responded within 1 month, they were contacted via SMS or e-mail two to three times. The total number of owners that received the questionnaire was 217.

The questionnaire comprised of 56 questions. The questionnaire is available as online supporting information (link to the questionnaire). A total of 49 variables were obtained from the questionnaire for the analysis. The variables are presented in Table S1.

Selection of cases and controls

Questionnaire information was obtained from 54% of the owners of eligible horses with a final number of horses being 117. Gastroscopy findings of these horses were reviewed by the author (JM) and divided into four groups: (1) EGGD \( (n = 43); \) (2) EGGD and ESGD \( (n = 40); \) (3) ESGD \( (n = 12); \) and (4) healthy \( (n = 22). \)

There were 79 horses for which gastroscopy was performed as an outpatient procedure and 38 hospitalized horses that underwent gastroscopy during their stay at the hospital \( (19 \text{ of } 38 \text{ on day } 2 \text{ of the hospitalization}; 14 \text{ of } 38 \text{ on day } 3; 3 \text{ of } 38 \text{ on day } 4; \text{ and } 2 \text{ of } 38 \text{ later than on day } 4, \text{ respectively}). \) The reason for horses’ hospitalization was colic in 34 of 38 cases.

The study population comprised 83 cases and 34 controls. A case was defined as a horse with endoscopic evidence of EGGD or EGGD and ESGD. All the different types of EGGD (hyperemic, hemorrhagic, fibrinosuppurative, ulcerated; depressed, flat, raised) were considered relevant for the purposes of the study (recorded as EGGD being present in our study), although hyperemia had to be subjectively moderate to be considered as relevant. EGGD lesions were not graded, as currently there is no valid system for that purpose. ESGD was recorded being present if the grade of the lesions was \( \geq 2 \) using the Equine Gastric Ulcer Council 0–4 scoring system. A control was defined as a horse with a healthy stomach \( (\text{ESGD grade } < 2) \) or a horse with endoscopic evidence of ESGD \( (\text{grade } \geq 2) \) without coincidental EGGD.

Statistical methods

The analyses were conducted for two differently defined response variables separately. The first analysis made use of the full dataset by comparing horses with EGGD (including both pure EGGD horses in group 1 and EGGD and ESGD horses in group 2, both coded 1) against all other horses (including pure ESGD horses in group 3 and healthy horses in group 4, both coded 0). The second analysis made use of a subset of the data, where horses with ESGD or both ESGD and EGGD were excluded, thus only comparing horses with pure EGGD \( (\text{group } 1, \text{ coded } 1) \) against healthy horses \( (\text{group } 4, \text{ coded } 0) \). We calculated

<table>
<thead>
<tr>
<th>Breed</th>
<th>Standard Error</th>
<th>Wald P-value</th>
<th>Bonferroni Corrected P-value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>Inverse of OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other breed versus Warmblood</td>
<td>0.947</td>
<td>0.005</td>
<td>0.015</td>
<td>0.072</td>
<td>0.011–0.458</td>
<td>13.9</td>
</tr>
<tr>
<td>Other breed versus Finnhorse</td>
<td>1.149</td>
<td>0.10</td>
<td>0.31</td>
<td>0.153</td>
<td>0.016–1.456</td>
<td>6.5</td>
</tr>
<tr>
<td>Warmblood versus Finnhorse</td>
<td>0.891</td>
<td>0.39</td>
<td>1.00</td>
<td>2.138</td>
<td>0.373–12.250</td>
<td>0.5</td>
</tr>
<tr>
<td>No. of caretakers (1–3 versus 4)</td>
<td>1.024</td>
<td>0.052</td>
<td>0.052</td>
<td>0.137</td>
<td>0.018–1.021</td>
<td>7.3</td>
</tr>
<tr>
<td>Previous colic (no versus yes)</td>
<td>0.648</td>
<td>0.14</td>
<td>0.14</td>
<td>2.606</td>
<td>0.732–9.274</td>
<td>0.4</td>
</tr>
<tr>
<td>Recurrent gastric ulcers (no versus yes)</td>
<td>1.348</td>
<td>0.14</td>
<td>0.14</td>
<td>0.138</td>
<td>0.010–1.931</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Table 1. Multivariable logistic regression analysis for EGGD present versus healthy horses and horses with ESGD in Finland between August 2013 and December 2014 (so called full data, \( n = 117 \)).
frequencies for the collected variables by case and control status for both the full data and the subset of the data. For these proportions the 95% confidence intervals (CI) were calculated with the EpiTools\textsuperscript{21} by means of Jeffrey’s method\textsuperscript{22} and they were used for preliminary descriptive comparisons between the cases and controls.\textsuperscript{16,17} Several recategorizations and simplifications were made to the independent variables to statistically model the data (as a result of low/zero frequencies). To avoid multicollinearity in the modeling process, the correlations between independent variables were tested with Spearman correlation method for ordinal variables, Fisher’s exact test and Kruskal–Wallis test for nominal variables. When one was continuous and the other nominal, and Wilcoxon Mann–Whitney if one was continuous and the other ordinal. To control for multicollinearity, only one of correlated variables was left in the modeling process.

In the first preliminary phase of the model building process potential risk factors were screened by crude logistic regressions with only one independent variable in the model at a time with the response variable. From these crude analyses the factors with \textit{P}-value \textless .10 for the overall effect were considered as potential risk factors for EGGD.

In the second phase of the model building process a stepwise process was used to build separate multivariable logistic regression models for full and subset data. In the iterative stepwise process, the following \textit{P}-values were used when choosing risk factors from a preliminary multivariable model of all potential risk factors (with \textit{P}-value \textless .10) to the actual to-be-final model: a significance level of 0.15 in the preliminary multivariable model was required to allow a variable into the actual model, and a significance level of 0.20 was required for a variable to stay in the actual multivariable model. For the resulting final models, \textit{P}-values \textless .05 were considered statistically significant but interesting variables with borderline significance were also left in the models. After receiving the final models from the stepwise modeling procedure for the full and subset data, all two-by-two interactions were tested (none were found). The potential confounding factors detected with a causal diagram (Appendix S1) were then entered one at a time to see their effect on the independent variables in the two models.

The goodness-of-fit of the final stepwise models was examined with a Hosmer and Lemeshow Goodness-of-Fit Test. Odds ratios (OR) were calculated to quantify and interpret the results. To help the interpretation of some OR values, the inverse of the OR was calculated in which case the comparison reversed. All statistical analyses were performed by SAS\textsuperscript{8} System for Windows, version 9.3.\textsuperscript{6}

### Results

Descriptive frequency results are shown in Table S1 for the full data and Table S2 for the subset of the data (Supporting Information).

1. Logistic regression results for the full dataset for the outcome of EGGD present (\( n = 83 \)) versus combination of healthy horses (\( n = 22 \)) and horses with ESGD (\( n = 12 \))

A total of 7 variables of the 49 variables screened at a crude level (Table S3, Supporting Information) were used in the building of the final multivariable model: breed, stable cleaning frequency, number of caretakers, amount of hay, number of riders, previous gastric ulceration, and previous colic. For full data, the results of the Hosmer and Lemeshow Goodness-of-Fit test were the following: Chi-Square 4.8256, DF 6, \textit{P}-value .57.

Equine gastric glandular disease was associated with breed and number of caretakers; a history of previous colic and recurrent gastric ulcers with \textit{P}-values \textless .20 but \textgreater .1 are shown in the model for discussion purposes (Table 1). Warmbloods had an increased risk of EGGD when compared with other breeds. Horses with four caretakers had increased risk of EGGD when compared with horses that had 1–3 caretakers. Horses that had experienced previous colic had decreased risk of EGGD compared to horses with no history of previous colic.

2. Logistic regression results for the subset of the data for the outcome of pure EGGD (\( n = 43 \)) versus healthy horses (\( n = 22 \))

A total of 7 variables of the 49 variables screened at a crude level were included in the multivariable modeling of the subset of the data comparing horses with just glandular lesions against healthy horses (Table S4, Supporting information), and similar results were obtained (Table 2). For subset of data, the results of the Hosmer and Lemeshow Goodness-of-Fit test were the following: Chi-Square 1.4437, DF 5, \textit{P}-value .92. Breed remained the most significant risk factor. Horses with three riders had increased risk of EGGD when compared with horses that had 1–2 riders. Horses that had sand in their colon had a decreased risk of EGGD compared with horses that did not have sand in their colon.

### Discussion

The most significant risk factor for EGGD in this study was the Warmblood breed. In a study population of Danish Warmbloods, an increased number and severity of lesions in the glandular mucosa were found compared to the squamous mucosa.\textsuperscript{8} Another study showed an increased prevalence of EGGD in nonracing riding horses when compared with ESGD.\textsuperscript{7} This is in stark contrast to other horse populations that have been studied, where the prevalence of ESGD has been consistently higher than that of EGGD, with the Thoroughbred breed in particular having been associated with a higher risk of developing ESGD.\textsuperscript{3,5,6,7,13,19} Although it is tempting to suggest that intrinsic differences in breed might play a potential role in the etiopathogenesis of EGUS (and EGGD in particular), it is prudent to consider the confounding effects that different management strategies such as feeding, stabling, and exercise routines might have on the eventual outcome in terms of risk, and in all likelihood, the breed effect on EGGD is multifactorial. For Warmbloods in their typical use the level of exercise is not very intense. Ischemia-reperfusion damage to the mucosa during exercise is therefore an unlikely mechanism for the development of EGUS in Warmbloods.\textsuperscript{23} This theory can be valid for racehorses, but unfortunately this cannot be assessed as they were not well represented in this study. The number of horses actively competing was quite low in our study population so the possible effect of discipline on
damage. In humans, increases in adrenocortical activity might be related to an increase in the incidence of gastric ulceration. The underlying pathophysiological mechanism is not well understood, but it is thought that stress (both psychological and physical) leads to oxidative stress within the stomach, resulting in elevated levels of reactive oxygen species.

In this study, increasing number of caretakers/riders was associated with an increased risk for EGGD. This could also potentially be attributed to the stress response caused by multiple people handling the horses. Unfortunately there are little data to support this theory. It has been shown that the effects of the daily relationship between the person who routinely takes care of the horse seemed to be involved in the reaction of the horse to a strange person. Horses appear to generalize their relationship with their caretaker to other people.

In another study, a horse which could be touched easily by a familiar human could also be touched and haltered easily by an unfamiliar human. This provides further evidence to support the fact that horses generalize their attitude toward different people—the so called “reactivity-to-humans” temperament trait. These findings would suggest that horses do not necessarily differentiate between different people handling them, but rather see all people as “reflections” of the main person handling them. However, horses are historically seen as “creatures of habit” who get fixed on their routines and are disturbed by changes in their daily life. An increased number of riders has been associated with a higher score on the emotionality axis. This could be interpreted that an increased number of riders can indeed be stressful for the horse. Further investigation on ethological aspects of the horse-human relationships, and in particular their effect on stress responses, is warranted.

In this study, sand in the colon had a protective role, although not significant \((P = .051)\) for EGGD. Sand enteropathy is a common problem for horses in Finland, other Nordic countries, and certain parts of the United States. Clinical signs of sand enteropathy can mimic those of gastric ulceration, as both clinical syndromes can present with colic and weight loss. It is not fully understood why certain horses consume sand or why the problem is scattered geographically. In Northern countries the pasture season is short (approximately 5 months); and for the remaining part of the year the horses are kept on sand/dirt paddocks and provided with supplementary roughage. It is possible that the horses that develop sand enteropathy are kept outside for a longer period of time each day. This should be less stressful for horses than stall confinement, however, the chances of horses consuming sand increases simultaneously. Based on this one could hypothesize that horses with sand enteropathy are less stressed by modern management (because of the fact that they are kept outdoors for longer periods of time) and thus are less prone to stress-related EGGD. In our study, however, an increase in time spent outdoors had no effect on developing EGGD. Unfortunately the relationship between time spent outdoors and sand enteropathy was not studied.

The number of recruited cases was reduced by the failure to acquire responses from all the owners of horses that met the inclusion criteria, and this could potentially have led to selection bias. This is a weakness inherent of all questionnaire-based studies, and could result in

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**Table 2.** Multivariable logistic regression analysis for EGGD versus healthy horses (a subset of the data, \(n = 65\)) in Finland between August 2013 and December 2014.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Standard Error</th>
<th>Wald P-value</th>
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<th>Odds Ratio</th>
<th>95% CI</th>
<th>Inverse of OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other breed versus Warmblood</td>
<td>1.152</td>
<td>.004</td>
<td>0.012</td>
<td>0.035</td>
<td>0.004-0.338</td>
<td>28.6</td>
</tr>
<tr>
<td>Other breed versus Finnhorse</td>
<td>1.499</td>
<td>.37</td>
<td>1.00</td>
<td>0.261</td>
<td>0.014-4.920</td>
<td>3.8</td>
</tr>
<tr>
<td>Warmblood versus Finnhorse</td>
<td>1.362</td>
<td>.14</td>
<td>0.43</td>
<td>7.368</td>
<td>0.510-106.347</td>
<td>0.1</td>
</tr>
<tr>
<td>Sand enteropathy (no versus yes)</td>
<td>0.851</td>
<td>.051</td>
<td>0.051</td>
<td>5.259</td>
<td>0.993-27.858</td>
<td>0.2</td>
</tr>
<tr>
<td>No. of riders 1–2 versus ≥3</td>
<td>1.339</td>
<td>.056</td>
<td>0.056</td>
<td>0.077</td>
<td>0.006-1.065</td>
<td>13.0</td>
</tr>
</tbody>
</table>

development of EGGD warrants further investigation—especially for Warmbloods, which are used for many different sports. It is possible that other breeds that were not represented in this study population could be at risk for developing EGGD, however, further studies targeting populations representing those breeds (eg, the Thoroughbred racing industry) would be necessary to quantify that risk.

In the above mentioned study Warmblood horses with severe EGGD had a higher cortisol response when exposed to a novel object test, and were thus considered to be more stress sensitive than other breeds. Interestingly in one EGUS prevalence study for show horses, horses with a nervous temperament had more gastric ulcers than the calmer ones (ESGD and EGGD not differentiated). These findings support the theory of psychological stress increasing the risk of developing EGUS and especially EGGD. When considering the effect of physiological stress on the development of EGUS in horses, there is even more robust evidence. Gastric glandular mucosal lesions were found in 40% of the foals stressed by severe illness in contrast to previous studies reporting very low occurrence of gastric mucosal lesions in neonates. The frequency of squamous mucosal lesions in this study was not different from previous reports. In laboratory animals, physical stress has been shown to increase gastrin concentrations and this is true with horses, too: strenuous exercise in horses has been shown to increase gastrin concentrations which can increase glandular secretion of hydrochloric acid, thus predisposing the mucosa to acid damage. In humans, increases in adrenocortical activity might be related to an increase in the incidence of gastric ulceration. The underlying pathophysiological mechanism is not well understood, but it is thought that stress (both psychological and physical) leads to oxidative stress within the stomach, resulting in elevated levels of reactive oxygen species.

### References

1. **Equine Glandular Gastric Disease**: 1273
2. **Table 2.** Multivariable logistic regression analysis for EGGD versus healthy horses (a subset of the data, \(n = 65\)) in Finland between August 2013 and December 2014.
reported risk for a particular variable that is either greater or smaller than the true risk. To improve our coverage, we could have expanded our question application methods from being only web based. There are still some people who, despite having access to the Internet, might not be comfortable in using such applications as our questionnaire. On the other hand, nowadays many people might use the Internet primarily with their smartphones/tablets and our questionnaire link was not modified to work smoothly on a mobile device.

Another source of selection bias could have occurred from the selection of the cases: as the clinical signs of EGGD are variable and can be missed by the handlers of the horses, there are probably many undiagnosed cases of EGGD, even in the hospital population. This bias would result in reported risk for a particular variable that is smaller than the true risk. In this study population 38/117 horses were hospitalized and underwent gastroscopy during their stay at the hospital. Previously a high prevalence of glandular lesions has been described in hospitalized horses. It is now possible to know whether the glandular lesions in the hospitalized horses did or did not form during hospitalization as there is only little information regarding the speed of glandular ulcer development. However, hospitalization was not a risk factor for EGGD in our statistical analysis.

When we studied effects of confounding factors—as detected with causal diagram and their ability to importantly change the odds ratios of the independent variables in the final models—by entering those factors into the final models, we noticed that, in general, the effects of the risk factors or protective factors in the final models increased substantially (Diarrhea, Forage location, and Hay amount were included as confounders for Previous colic and Forage location and Amount of hay for Recurrent gastric ulcers in the full model; and Forage location and Sand paddock were included as confounders for Sand enteropathy in the subset model). This indicates that the associations found in our study seem to be underestimations. We preferred, however, to present more conservative effects without including the confounders. In addition, we cannot ignore the possibility of type-1 errors for the found new associations with the EGGD; they remain for future studies to confirm or disprove.

In a retrospective study there is always a risk for recall bias. Nevertheless, in this pilot study we considered it important to obtain information for an extended time period in past as the time for EGGD to develop is not known. For the majority of the questions the response rates were good. Unfortunately, for the questions concerning the amounts of forage and concentrates provided, the response rate was lower and some of the answers were so imprecise that they had to be excluded. In particular, the amounts of concentrates provided were not clearly stated by many owners. This could be related to the format of the question which was an open-ended question. If we were to use this questionnaire in the future for a larger study, this would be one point for improvement. As the response rate for these questions was lower, the value of the questionnaire for evaluating potential feeding-related risk factors is questionable.

Conclusions

Risk factors identified in this study suggest that Warmbloods are predisposed to EGGD and that the horse having multiple handlers or riders could increase the risk of EGGD. This is the first pilot study focusing on risk factors for EGGD and the study design could be used for bigger populations of horses in search of generalization of these results.

Footnotes

a SAS Institute Inc., Cary, NC

Acknowledgments

The authors thank Heidi Harinen for her technical assistance and Jouni Junnila and Sofia Männikkö for their assistance with the statistical analysis.

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

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

References

10. Luthersson N, Nielsen K, Harris P, Parkin T. The prevalence and anatomical distribution of equine gastric ulceration...


Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Questionnaire variables and their proportions for descriptive purposes.

Table S2. Questionnaire variables and their proportions, subset of data.

Table S3. Crude preliminary logistic regression analyses where only one independent variable in the model at a time with the response variable EGGD present versus healthy horses and horses with ESGD (full data).

Table S4. Crude preliminary logistic regression analyses where only one independent variable in the model at a time with the response variable pure EGGD versus healthy horses (a subset of the data).

Appendix S1. EGGD- Equine Glandular Disease.