Diagnostic accuracy of post-ACTH challenge salivary cortisol concentrations for identifying horses with equine glandular gastric disease[1]

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ABSTRACT: The aims of this study were to better characterize the adrenal response to i.v. adrenocorticotropic hormone (**ACTH**) in horses with and without gastric disease and to validate and simplify the ACTH stimulation test by determining the diagnostic accuracy of six consecutive sampling time points after ACTH administration for equine glandular gastric disease (**EGGD**) and equine squamous gastric disease (**ESGD**). Twenty-six endurance and eventing horses without clinical disease [Sport Horse Population (**SHP**)] and an independent population of 62 horses [General Population (**GP**)] were grouped by gastroscopic findings (no/mild vs. moderate/severe EGGD, grade 0–1 vs. 2–4 ESGD, respectively) and underwent an ACTH stimulation test. Salivary cortisol (ng/mL) was analyzed before and 30, 60, 90, 120, and 150 min after i.v. injection of 1 µg/kg BW synthetic ACTH1-24. The association between having moderate or severe EGGD or ESGD and the amount of salivary cortisol was analyzed by means of receiver-operating characteristic (**ROC**) analysis. The following explanatory variables were considered: cortisol values for every time point, the area under the curve (**AUC**)—including all time points and corrected for the baseline—and the partial areas under the curve AUC0-90 and AUC90-150. Sampling after 60 min had highest association with moderate/severe EGGD. The diagnostic potential of the ACTH test was higher for the SHP [sensitivity 100% (95% CI 54% to 100%), specificity 75% (95% CI 51% to 91%), ROC-AUC 91% (95% CI 69% to 98%), 1-sided *P*-value < 0.001] than for the GP [sensitivity 75% (95% CI 48% to 93%), specificity 52% (95% CI 37% to 67%), ROC-AUC 68% (95% CI 51% to 79%), 1-sided *P*-value = 0.0064]. There were, however, no significant associations with ESGD. The superiority of sampling after 60 min suggests that the initial release of cortisol rather than its peak or the AUC are relevant regarding EGGD. Even though the wide confidence intervals and thus the lack of diagnostic accuracy do not presently support clinical use, characterization of the adrenal response to an ACTH stimulus improves the understanding of EGGD pathophysiology and its relation to stress.

Key words: ACTH stimulation test, chronic stress, cortisol, EGGD, equine, receiver-operating characteristics

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INTRODUCTION

Assessment of chronic stress in equine medicine remains challenging. Stress from training (Linden et al., 1991; Strzelec et al., 2011), transportation (Fazio et al., 2008; Schmidt et al., 2010), competition (Becker-Birck et al., 2013),

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isolation (Alexander et al., 1988; Strand et al., 2002; Harewood and McGowan, 2005) or social rivalry in groups (Alexander and Irvine, 1998) has long been suspected to play a role in behavioral and medical problems. Several studies suggest an association between stress and equine glandular gastric disease (**EGGD**) (Furr et al., 1992; Malmkvist et al., 2012; Scheidegger et al., 2017). In comparison to equine squamous gastric disease (**ESGD**), EGGD is still poorly understood (Sykes et al., 2015).

Different approaches including the assessment of behavioral (Bachmann et al., 2003; Rietmann et al., 2004), autonomic nervous system (Bachmann et al., 2003; Rietmann et al., 2004) and neuroendocrine reactions (Alexander and Irvine, 1998; Briefer Freymond et al., 2015), specifically regarding the hypothalamic pituitary adrenal (**HPA**) axis, have been used to determine stress responses in horses. The HPA-axis plays a critical role in chronic stress (Mormède et al., 2007) and has broad and long-lasting effects on the body (Moberg and Mench, 2000).

Recently, our group was able to show that moderate to severe glandular gastric lesions are accompanied by an exaggerated release of cortisol from the adrenal cortex in response to an adrenocorticotropic hormone (**ACTH**) stimulus (Scheidegger et al., 2017). Currently, however, the ACTH stimulation test is time-consuming and requires repeated sampling over a period of several hours. Thus, the objectives of the present study were to better characterize the adrenal response to an ACTH-challenge in horses with and without gastric disease and to determine the time point with the best diagnostic accuracy for EGGD and ESGD.

MATERIALS AND METHODS

Sample Populations

All experimental procedures were authorized by the cantonal committee of the city of Bern of animal protection and welfare issues (Approval number: 27608/BE26/16). Two different populations were investigated in the same manner. The first population [Sport Horse Population (**SHP**)] consisted of 26 high level endurance and eventing horses. Their data was used retrospectively. The cortisol response to ACTH in relation to observed gastric lesions was described by Scheidegger et al. (2017), but no receiver-operating characteristic (**ROC**) analyses for determination of best sampling time points for detection of gastric disease were performed. The second group [general population (**GP**)] was composed of 62 horses that were presented prospectively to the Swiss Institute of Equine Medicine ISME for a gastric endoscopic examination between May 23, 2013 and February 9, 2017.

Horses of both populations arrived at least 1 d before the examination and were housed in individual box stalls with wood shaving bedding. Horses were fasted for 12 h before gastroscopy to ensure complete visualization of all areas of the stomach. Horses were not allowed to drink from the time of the injection of the ACTH analogue until the end of the test, in order to avoid dilution of the saliva.

Gastroscopy

For the endoscopic examination of the stomach, all horses were sedated with detomidine (Equisedan, Dr. E. Graeub AG, Bern, Switzerland; 0.01 mg/kg BW) and butorphanol (Morphasol, Dr. E. Graeub AG, Bern, Switzerland; 0.1 mg/kg BW) and restrained with a twitch if necessary. The video endoscope (Anklin AG, Binningen, Switzerland) was passed into the stomach. Air was insufflated, then the stomach was explored systematically, first the fundus, then the greater and lesser curvatures, the antrum and the pylorus. All images and videos were captured during the examination. Grading of the videos of the SHP had already been performed by two of the authors (A.R. and M.S.) for the previous publication (Scheidegger et al., 2017). The videos of the GP were evaluated by one of these authors (A.R.) who was unaware of the outcome measures of the ACTH stimulation test. According to the European College of Equine Internal Medicine (ECEIM) Consensus Statement (Sykes et al., 2015), glandular lesions (EGGD) were described according to their anatomical localization, distribution and appearance based on the extent of the lesions (no, mild, moderate or severe lesions) and squamous lesions (ESGD) were scored using the Equine Gastric Ulcer Council scoring system [ranging from 0 (epithelium is intact, no appearance of hyperkeratosis) to 4 (extensive lesions with areas of apparent deep ulceration)].

ACTH Stimulation Test

An ACTH stimulation test was performed on all horses in a similar manner. As previously described by Peeters et al. (2011) and Stewart et al. (2011), a dose of 1 μg/kg BW synthetic ACTH1-24 (Synacthen tetracosactidum 0.25 mg/mL equivalent to 25 IU/mL; Novartis, Vilvoorde, Belgium) was chosen for the test and administered i.v. Saliva samples were collected with salivettes (Sarstedt, Nuembrecht, Germany) before (0 min – baseline) and 30, 60, 90, 120, and 150 min after ACTH application. A swab was placed into the mouth of the horse with help of a clamp and maintained there for at least 40 s. When it was completely soaked with saliva, the swab was replaced into the salivette. Salivettes were centrifuged at room temperature for 10 min at 100.62 × *g* and then stored at −20 °C until the analysis.

Salivary cortisol concentrations (ng/mL) were determined using a competitive enzyme immunoassay (cELISA, Salimetrics, Newmarket, United Kingdom), previously validated for use in horses (Scheidegger et al., 2016; Scheidegger et al., 2017).

Statistical Analysis

Statistical analysis of the data was performed with the software package NCSS10 (NCSS Statistical Software, Kaysville, UT, USA) and the R-interface of RStudio (RStudio Team, 2016, Boston, MA, USA). Concerning EGGD, horses without abnormal gastroscopic findings or only mild lesions were combined in one group and horses with moderate and severe glandular lesions in another. Since mild hyperkeratosis (ESGD grade 1) might also occur because of fasting (Murray, 1994; Scheidegger et al., 2017), horses with ESGD grade 0 to 1 and horses with ESGD grade 2 to 4 were grouped together, respectively. Cortisol concentrations (ng/mL) of all time points were analyzed after subtraction of the baseline value.

Characterization of the AUC of the cortisol response curve (NCSS10). When plotting the cortisol concentration along the sampling time points (0 to 150 min), the area under the cortisol curve (**AUC**) corrected for the baseline represented the total increase in cortisol concentration during the entire sampling period. Additionally, the AUC was calculated separately for the partial curves between time point 0 and 90 (**AUC0-90**) and between 90 and 150 (**AUC90−150**) in order to investigate the initial increase of cortisol (AUC0-90) vs. following the plateau and decrease (AUC90-150), respectively. The Shapiro-Wilk test as well as visual inspection of the histograms and quantile-quantile plots were used to check for normal distribution of the variables (Salivary cortisol at the time points 0, 30, 60, 90, 120, 150 and as well the AUC). Salivary cortisol concentrations were normally distributed at all time points. The AUC for the SHP and of the time points 90 and 150 min for the GP also showed a normal distribution. The variability of cortisol concentrations for the GP was much higher, and normality was rejected for the time points 30, 60, and 120 min and the respective AUC. Pearson's and Spearman's correlation analyses between the salivary cortisol AUC and the cortisol values at the different time points (0 to 150 min) during the 2.5 h recording period of the ACTH stimulation test were performed, in order to determine the time point which best correlated with the AUC.

Association of EGGD/ESGD and cortisol concentrations (ROC analyses) (NCSS10). For every time point, the association between having moderate or severe EGGD or ESGD and the amount of salivary cortisol was analyzed by means of ROC analyses. Both, the cortisol concentrations at each time point, as well as the AUC and the AUC0-90 and AUC90-150 were considered as explanatory variables. The best cut-off value was then established from the ROC curves, which is the value that maximizes sensitivity and specificity to identify horses with moderate to severe EGGD or ESGD grade 2 to 4, respectively. Best cut-off values were selected manually for each time point. 1-sided *P*-value as well as the estimated area under the ROC curve (**ROC-AUC**) and its standard error (**SE**) were determined. The 1-sided *P*-value confirmed that the AUC is bigger than 50% (50% meaning zero diagnostic value). Furthermore, 95% confidence intervals (95%CI) were calculated for sensitivity, specificity and the ROC-AUC by considering the exact CI of a proportion. Data of the SHP and the GP were analyzed separately and compared afterwards. Significance was set at $P < 0.05$.

Analysis of variables potentially affecting the HPA (RStudio). In order to control for individual horse characteristics, which might confound the association between EGGD and cortisol concentrations, a multivariable analysis was performed in RStudio. For the SHP, linear mixed models were calculated to assess effects of sex (mare/gelding), breed (Warmblood/Franches-Montagnes/pony/other), age, EGGD and ESGD on cortisol concentrations at baseline and 60 min after ACTH administration. The same was done for the GP. There, however, clinical signs (healthy/recurrent colic episodes/acute colic/weight loss/other) were included in the models as an additional explanatory variable (function lm). Residuals of the models were checked visually via scatterplots for independence and via quantile-quantile-plots and histograms for normal distribution. The models were tested for homoscedasticity of variance via visual inspection and Breusch-Pagan test (package car, function ncvTest). Linear model assumptions were best met after square-root transformation of the respective response variable (baseline cortisol concentrations or cortisol concentrations after 60 min, respectively). Variables of the models were checked for multicollinearity via use of the variance inflation factor (package car, function vif). It was adequate in all cases. Initially, all variables were entered in the models, which were then reduced by stepwise backward selection (function step). The fit of the models was measured by Akaike's Information Criterion (**AIC**). The model with the lowest AIC was kept as the final model.

RESULTS

Sample Populations

Data are reported as mean ± SD (range). The age of the SHP was 11 ± 3 (6 to 19) years and the bodyweight 484 ± 65 (368 to 632) kg, respectively. Breeds were mainly Warmbloods (n = 15) and Arabians (n = 10). One Franche-Montagne was included. The details have been previously described (Scheidegger et al., 2017).

For the GP, the age was 11 ± 5 (3 to 27) years and the bodyweight 522 ± 145 (92 to 820) kg. Breeds included Warmbloods (n = 35), Franches-Montagnes (n = 6), ponies (n = 6), Quarter Horses (n = 3), Pura Raza Españolas (n = 2), Thoroughbreds (n = 3), one Friesian, one Comtois, one Shire, one Percheron, one Pinto, one Tinker, and one mixed breed horse. Some of them were clinically healthy (n = 12), others showed clinical signs [recurrent colic episodes (n = 19), acute colic (n = 20), weight loss (n = 5), and other gastrointestinal signs (n = 6)]. Four out of twelve clinically healthy horses were owned by the clinic, the other eight were presented by their owners in order to participate in the study.

Gastroscopy

The squamous mucosa could be fully observed in all horses. In most cases, residual fluid covered the ventral part of the glandular body, however, the glandular part of the greater and lesser curvatures as well as the pyloric antrum and the pylorus could be visualized and evaluated in all horses. Endoscopic gastric examination revealed that 11 horses of the SHP (42%) and 28 horses of the GP (45%) had no gastric disease (EGGD no or mild and ESGD grade 0 or 1). Six horses of the SHP (23%) and 16 of the GP (26%) had moderate or severe EGGD. Thirteen horses of the SHP (50%) and 27 of the GP (44%) had lesions in the squamous part of the stomach between grades 2 and 4.

ACTH Stimulation Test—AUC Evaluation, ROC Analyses, and Analysis of Variables Affecting the HPA

In the majority of horses, the maximum cortisol concentration was reached at 120 min (SHP n = 16/26, 62%; GP n = 23/62, 37%) or 150 min (SHP n = 5/26, 19%; GP n = 28/62, 45%) postinjection. For the SHP, Pearson's correlation coefficient revealed associations ($P < 0.001$) between the AUC and cortisol concentrations at all time points. The cortisol at time point 120 min showed the highest correlation value: 0.92. For the GP, Spearman's correlation coefficient also revealed correlations between the AUC and all time points ($P < 0.001$; $r > 0.7$). Data of the sampling time point 120 min presented again the highest correlation ($r = 0.93$) (Table 1).

Based on the ROC analysis of the SHP, sampling 60 min after ACTH injection had the best diagnostic accuracy for detection of moderate or severe EGGD (Table 2 and Table 3, a: SHP). Analysis of the GP independently confirmed that sampling after 60 min provided best results (Table 2 and Table 3, b: GP). Even though P-values were significant, the 95%CI of sensitivity, specificity and

Table 1. Comparison of the correlation coefficients of the different time points and the AUC for the SHP (Pearson's correlation coefficient) and GP (Spearman's correlation coefficient), respectively

	T1 (30 min)	T2 (60 min)	T3 (90 min)	T4 (120 min)	T5 (150 min)	AUC
Pearson's correlation coefficient for the SHP						
AUC	0.72932	0.84312	0.91741	**0.92197**	0.7972	1.00000
P-value	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000
Spearman's correlation coefficient for the GP						
AUC	0.70328	0.80957	0.85680	**0.94146**	0.80915	1.00000
P-value	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

AUC = area under the curve; GP = the general population; SHP = sport horse population. Bold value signifies P-value = 0.00.

Table 2. Results of the ROC analyses of two equine populations (SHP and GP), 60 min after i.v. injection of an ACTH analogue

Population	Cut-off	Se	95% CI	Sp	95% CI	ROC-AUC	95% CI	ROC AUC's SE	*P*-value
SHP	4.92	100%	54–100%	75%	51–91%	91%	69–98%	0.06001	0.0000
GP	4.04	75%	48–93%	52%	37–67%	68%	51–79%	0.07043	0.0064

For each population, the cut-off value (Cut-off) with best sensitivity (Se) (including its 95% CI), specificity (Sp) (including its 95% CI), area under the ROC-curve (ROC-AUC) [including its 95% CI and its standard error (ROC-AUC's SE)] and 1-Sided *P*-value (*P*-value) is given.

ACTH = adrenocorticotropic hormone; CI = confidence interval; GP = the general population; ROC = receiver-operating characteristic; SHP = sport horse population

Table 3. Comparison of the results of the ROC analyses from (a) SHP and (b) GP at all time points [T1 (30 min) – T5 (150 min)] and AUC, AUC0–90 and AUC90–150

(a) SHP

Time Point	Cut-off	Se	95% CI	Sp	95% CI	ROC-AUC	95% CI	ROC AUC's SE	*P*-value
T1 (30 min)	2.92	83%	36–100%	75%	51–91%	78%	51–91%	0.09656	0.0017
T2 (60 min)	4.92	100%	54–100%	75%	51–91%	91%	69–98%	0.06001	0.0000
T3 (90 min)	7.14	83%	36–100%	80%	56–94%	87%	62–96%	0.07553	0.0000
T4 (120 min)	7.26	83%	36–100%	60%	36–81%	80%	51–93%	0.09732	0.0010
T5 (150 min)	7.05	83%	36–100%	70%	46–88%	78%	51–91%	0.09558	0.0020
AUC	24.4	100%	54–100%	70%	46–88%	86%	62–95%	0.07523	0.0000
AUC 0–90	9.53	100%	54–100%	65%	41–85%	89%	64–97%	0.06945	0.0000
AUC 90–150	–6.47	50%	12–88%	40%	19–64%	30%	7–49%	0.11010	0.0402

(b) GP

Time Point	Cut-off	Se	95% CI	Sp	95% CI	ROC-AUC	95% CI	ROC AUC's SE	*P*-value
T1 (30 min)	1.40	75%	48–93%	51%	36–66%	64%	44–78%	0.08299	0.0455
T2 (60 min)	4.04	75%	48–93%	52%	37–67%	68%	51–79%	0.07043	0.0064
T3 (90 min)	7.23	56%	30–80%	59%	43–73%	62%	46–75%	0.07291	0.0468
T4 (120 min)	8.40	63%	35–85%	65%	50–79%	65%	48–78%	0.07582	0.0233
T5 (150 min)	7.25	56%	30–80%	54%	39–69%	61%	43–75%	0.08103	0.0820
AUC	25.12	63%	35–85%	61%	45–75%	66%	49–79%	0.07539	0.0231
AUC 0–90	9.30	69%	41–89%	57%	41–71%	68%	51–80%	0.07284	0.0073
AUC 90–150	−5.12	50%	25–75%	61%	45–75%	47%	30–61%	0.08000	0.0086

For each variable, the cut-off value (Cut-off) with best sensitivity (Se) (including its 95% CI), specificity (Sp) (including its 95% CI), area under the ROC-curve (ROC-AUC) [including its 95% CI and its SE (ROC-AUC's SE)] and 1-Sided *P*-value (*P*-value) is given.

AUC = area under the curve; CI = confidence interval; GP = the general population; ROC = receiver-operating characteristic; SHP = sport horse population.

ROC-AUC were rather wide, indicating considerable uncertainty of the estimates. The AUC0-90 performed better than AUC90-150. The ROC-curve of AUC90-150 did not discriminate between healthy horses and horses with EGGD.

In contrast, the ROC-analyses of the different time points did not reveal any cut-off values that yielded significant associations with ESGD scores.

Results of the linear mixed models are presented in Table 4. For both populations, EGGD was the only remaining variable in the final model with cortisol concentrations at 60 min as the outcome variable. However, EGGD was only significantly associated with cortisol concentrations 60 min after ACTH administration in the SHP.

Baseline cortisol concentrations were significantly associated with breed and clinical signs in the GP. The model for baseline cortisol concentrations in the SHP was marginally nonsignificant, with EGGD as the only variable remaining in the model.

DISCUSSION

The aim of the present study was to better characterize the adrenal response to i.v. administered ACTH and to validate and simplify the ACTH

Table 4. Results of the linear mixed models evaluating the association between cortisol concentrations at baseline, and 60 min after ACTH administration with the presence of EGGD, ESGD, and horse characteristics in two different horse populations (SHP and GP)

Model	Variable	Parameter estimate	Standard error	*P*-value
Baseline cortisol in SHP		EGGD:		
	EGGD (reference: No)	-	-	-
	EGGD yes	−0.3375	0.16872	0.0569
Baseline cortisol in GP		Breed:		
	Warmblood (reference)	-	-	-
	Franches-Montagnes	−0.07293	0.09036	0.42314
	Pony	−0.29398	0.08999	0.00189
	Other	−0.14056	0.06319	0.03032
		Clinical signs:		
	Healthy (reference)	-	-	-
	Recurrent colic episodes	0.23698	0.07855	0.00389
	Acute colic	0.17442	0.07486	0.02357
	Weight loss	−0.04985	0.10072	0.62264
	Other gastrointestinal signs	0.17906	0.09956	0.07769
Cortisol 60 min after ACTH stimulation in SHP		EGGD:		
	EGGD (reference: No)	-	-	-
	EGGD yes	2.52	0.6839	0.00116
Cortisol 60 min after ACTH stimulation in GP		EGGD:		
	EGGD (reference: No)	-	-	-
	EGGD yes	0.3325	0.16973	0.0548

ACTH = adrenocorticotropic hormone; EGGD = equine glandular gastric disease; ESGD = equine squamous gastric disease; GP = the general population; SHP = sport horse population

stimulation test by determining the diagnostic accuracy of six consecutive sampling time points after ACTH administration for gastric disease.

We found that cortisol concentrations measured at 120 min correlated best with the total cortisol increase after the ACTH stimulation test. As found in previous studies that used the same dosage of i.v. ACTH, time point 120 min also represented the maximum of the curve in most horses (Bousquet-Mélou et al., 2006; Peeters et al., 2011; Scheidegger et al., 2016; Scheidegger et al., 2017). Our results support that sampling after 120 min constitutes a good predictor of the total cortisol increase. Regarding EGGD, however, the present results of the ROC analyses showed that salivary cortisol measurement from samples at the second time point, 60 min after ACTH administration, was superior to any cortisol value at any other time point in detecting EGGD. Results of the ROC analysis of cortisol values at 60 min were also better than the results of the evaluation of the AUC and the AUC0-90.

Currently, gastroscopy is considered the gold standard for detection of gastric disease against which all potentially new diagnostic tests need to be compared (Hewetson et al., 2017). The ROC curve is a widely accepted method for describing the accuracy of a diagnostic test and is obtained by plotting the true-positive rate (sensitivity) on the y-axis against the false-positive rate (1-specificity) on the x-axis (Greiner et al., 2000; Akobeng, 2007). The AUC of each ROC curve (ROC-AUC) thereby presents the overall accuracy of the time point, meaning that 100% indicates perfect discrimination between horses with and without EGGD or ESGD, respectively. Apart from this, according to an arbitrary guideline, the ROC-AUC can be used to differentiate between a test with high accuracy (90%<AUC<100%), moderate accuracy (70%<AUC≤90%), less accuracy (50%<AUC≤70%) and a noninformative test (AUC = 50%) (Swets, 1988). Values for ROC-AUC were also highest for sampling after 60 min and for the AUC0-90. The point estimator of the ROC-AUC for the SHP at 60 min was higher (91%) than that of the GP (68%).

Interestingly, sampling at 60 min after injection did not represent the maximum or plateau of the adrenal response curve, but rather its ascent. In comparison to the AUC, analysis of the AUC0-90 led to slightly improved results. Results of the sampling at 60 min, however, still showed maximal values for sensitivity, specificity and ROC-AUC. These findings may indicate that the initial release of cortisol from the adrenal cortex in response to an ACTH stimulus rather than its maximal expression is relevant regarding EGGD and potentially other stress-related disorders.

The analysis of the new independent GP confirmed the results of the SHP. Importantly, it served as a replication population for the SHP, verifying that the reported identification of the best sampling time point is robust and consistent. Comparison of the results of both groups showed, however, higher percentages for sensitivity, specificity and ROC-AUC in the SHP. This discrepancy is not surprising considering the heterogeneity of the GP. In contrast to the SHP, which was composed of competition horses that did not manifest clinical signs, a limitation of the GP was the diverse combination of different breeds, ages and activity levels with some animals manifesting clinical signs. Indeed, examination of potential influence of horse characteristics on baseline cortisol and the cortisol response in multivariable models showed that breed and concurrent colic episodes had an effect on baseline cortisol concentrations. The cortisol response at 60 min post-ACTH injection, however, was only associated with EGGD. None of the potential confounders showed a significant effect on the adrenal response to ACTH stimulation, indicating that the ACTH stimulation test results are more robust (i.e., not affected by these common potential confounders) than cortisol baseline values. Furthermore, the wide 95% CIs and overall lower specificity indicate that its predictive value is limited. Confidence intervals were not always presented in previous clinical studies, but are very important to decide whether a particular diagnostic test is useful or not.

Based on the ROC analyses, we showed that the ACTH stimulation test has no diagnostic value for detecting ESGD. We had already reported this difference in the adrenal response between EGGD and ESGD in the SHP (Scheidegger et al., 2017); the new data from the GP confirm this observation. The ACTH test's lack of diagnostic value for ESGD is the most important reason, why it is an imperfect surrogate marker and cannot replace gastroscopy for evaluation of gastric disease. In addition, the maximal specificity of the test for EGGD was rather low (75%). Nevertheless, the results confirm the importance of clearly differentiating between squamous and glandular lesions as suggested by the ECEIM Consensus Statement (Sykes et al., 2015). As the distinction between EGGD and ESGD was defined only recently, further research concerning the pathophysiology, risk factors, and treatment options for glandular gastric lesions is needed. Interestingly, horses with other pathologies can also show altered responses to ACTH administration. In a study by Briefer Freymond et al. (2015), crib-biting horses had an increased response to i.v. ACTH. Apparently, an exaggerated cortisol release seems to be associated with different medical problems, such as stereotypies and EGGD, which are typically thought to be related to chronic stress. It remains to be elucidated, whether the test has a diagnostic value for other potentially "stress-induced disorders," such as prolonged recovery times or subclinical infections following horse shows and long transports.

We conclude that horses with moderate or severe EGGD had not only a larger (Scheidegger et al., 2017), but also a quicker increase in cortisol concentration following ACTH stimulation with best diagnostic accuracy at 60 min postinjection. The detailed analysis of the adrenal response to an ACTH stimulus in the present study, in addition to other recently published studies (Malmkvist et al., 2012; Mönki et al., 2016; Scheidegger et al., 2017), leads to further improvement of the understanding of the pathophysiology of EGGD and its relation to stress.

Conflict of interest statement. None declared.

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