



Section

Fields (of activity)

Development of a „stress test“ in horses (adrenal cortisol response to ACTH) and validation of predictive value for gastric ulcers and other stress-induced health problems, particularly in sport horses

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Key words

Chronic stress, ACTH stimulation test, Cortisol, Equine Squamous Gastric Disease, Equine Glandular Gastric Disease, Sport horse, Personality, immunoassay, liquid chromatography tandem mass spectrometry

Aim of the study

This project was performed to: **(1)** evaluate the use of the adrenocorticotrophic hormone (ACTH) stimulation test as a tool for diagnosis of Equine Squamous Gastric Disease (ESGD) and Equine Glandular Gastric Disease (EGGD) by determining the best time point and cut-off to maximise sensitivity and specificity of salivary cortisol values after ACTH injection; **(2)** determine risk factors for ESGD and EGGD; **(3)** compare the adrenal response to external ACTH of elite and amateur sport horses of different disciplines and to investigate the effect of different breeds, demographic, management and personality traits; **(4)** establish liquid chromatography tandem mass spectrometry (LC-MS/MS) for determination of equine plasma and saliva cortisol; **(5)** establish total and free cortisol as well as CBG measurements in equine plasma in order to better characterise the exaggerated response in stress-susceptible individuals.

Material and methods

(1) Twenty-six endurance and eventing horses without clinical complaints and an independent population of 62 patients 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 was analyzed before and 30, 60, 90, 120 and 150 minutes after intravenous injection of exogenous ACTH. Receiver-operating-characteristic (ROC) analyses of all time points were performed.

(2) A total of 23 clinically healthy horses and 50 horses with clinical signs underwent a gastroscopy and a person who was familiar with the horse completed a questionnaire about demographic, management and personality traits. Independent masked reviewers placed the horses into one of three groups based on the clinical signs and history: 1) healthy; 2) acute colic; 3) recurrent colic; 4) other disorders. A risk factor analysis was performed using multivariable logistic regression modelling.

(3) A total of 149 healthy elite (n=94) and amateur (n=54) sport horses underwent an ACTH stimulation test and a person who was familiar with the horse completed the same questionnaire as in (2). Linear models were calculated to assess associations between the questionnaire data and salivary cortisol 60 and 90 minutes after ACTH stimulation.

(4) Plasma: Eleven clinically healthy horses underwent an ACTH stimulation test. The plasma samples at 0 minutes before and 60 minutes after stimulation were analyzed for their content of several steroids using LC-MS/MS. Saliva: Nine clinically healthy horses underwent an ACTH stimulation test and salivary cortisol was assessed before and 60 minutes after stimulation by means of an immunoassay and LC-MS/MS for method comparison.

(5) Two different methods were investigated for determination of free cortisol before and after ACTH stimulation: An indirect ligand-binding ultrafiltration method and equilibrium dialysis, coupled to LC-MS/MS. CBG was analyzed with an immunoassay.

Results and significance

(1) Salivary cortisol 60 minutes after ACTH injection showed best agreement with moderate/severe EGGD. Accordingly, the initial release of cortisol rather than its peak or total increase appeared to be relevant regarding EGGD. There were, however, no significant associations with ESGD. The result may improve the understanding of the pathophysiology of EGGD and its relation to stress.

(2) The frequency of feeding, stereotypies and clinical signs before gastroscopy were identified as risk factors for ESGD. The findings of this study, as well as the results of (1), support the hypothesis that ESGD and EGGD should be considered as different entities.

(3) There was no difference between the adrenal response to ACTH of elite and amateur sport horses. The competition discipline and personality traits had no effect either. However, the breed and several management factors, such as 'the number of riders', 'the time spent outside' or 'group housing' affected the adrenal responsiveness. This suggested that optimizing husbandry conditions may be more important for improving equine welfare than changing the horses' use.

(4) Plasma: LC-MS/MS represented a viable method to evaluate multiple steroid metabolites, including cortisol in equine plasma samples. Eleven-deoxycorticosterone, 11-deoxycortisol, and corticosterone showed a more pronounced response to ACTH when compared to cortisol. Accordingly, these three metabolites may serve as new, potentially more sensitive biomarkers for stress assessment in horses than cortisol. Saliva: Comparison of immunoassay and LC-MS/MS measurements for salivary cortisol values before stimulation showed poor agreement between both methods. Sixty minutes after stimulation, immunoassay measurements led to higher outcome values than LC-MS/MS, but the results were reasonably correlated. In agreement with the literature, LC-MS/MS may provide more accurate outcome measures, particularly for baseline cortisol concentrations close to the limit of detection of the ELISA.

(5) Both methods worked to demonstrate elevated free cortisol concentrations following ACTH administration. Compared to the ligand-binding ultrafiltration method, the use of equilibrium dialysis in combination with LC-MS/MS is new in horses and allows direct rather than indirect assessment of free cortisol. Interestingly, CBG concentrations remained the same before and after ACTH stimulation. A decrease in CBG concentrations, which was expected with the increasing proportion of free cortisol, may have only occurred after the sampling period of the study. This remains to be further investigated.

Publications, posters and presentations

Publications:

- (1) Sauer, F.J.; Bruckmaier, R.M.; Ramseyer, A.; Vidondo, B; Scheidegger, M.D.; Gerber, V. (2018) Diagnostic accuracy of post-ACTH challenge salivary cortisol concentrations for identifying horses with equine glandular gastric disease. *Journal of Animal Science*. Volume 96, Issue 6, Pages 2154 – 2161.
- (2) Sauer, F.J.; Riemer, S.; Ramseyer, A.; Axiak Flammer, S.; Bütikofer, L.; Gerber, V. Assessment of clinical signs, husbandry and personality factors on risk of gastric disease in horses. *Submitted for publication: Journal of Veterinary Internal Medicine*.
- (3) Sauer, F.J.; Hermann, M.; Ramseyer, A.; Burger, D.; Riemer, S. and Gerber, V. (2019). Effects of breed, management and personality on cortisol reactivity in sport horses. *PloS One*. Volume 14, Issue 12, Pages e0221794.
- (4) Kirchmeier, A.; van Herwaarden, A.E.; van der Kolk, J.H.; Sauer, F.J.; Gerber, V. (2019). Plasma steroid profiles before and after ACTH stimulation test in healthy horses. *Domestic Animal Endocrinology*. Pages 106419

- (4) Sauer, F.J.; Gerber, V.; Frei, S.; Bruckmaier, R.M.; Grössl, M. Salivary cortisol measurement in horses: Immunoassay or LC-MS/MS? *Domest Anim Endocrinol.* 2020:106445.
- (5) Schwinn, A-C. and Sauer, F.J.; Gerber, V.; Bruckmaier, R.M.; Gross, J.J. (2018) Free and bound cortisol in plasma and saliva during ACTH challenge in dairy cows and horses. *Journal of Animal Science.* Volume 96, Issue 1, Pages 76-84.

Posters:

- (4) Kirchmaier, A. (2019) Steroidprofile vor und nach ACTH Stimulation bei Pferden. April 10, 2019; Netzwerktagung, Avenches, Switzerland.
- (5) Schwinn, A-C. (2017) Binding and distribution of cortisol in bovine and equine saliva and plasma during ACTH challenge. August 28 – September 1, 2017; Annual Meeting of the European Federation of Animal Science (EAAP), Tallinn, Estonia.

Presentations:

- (General) Gerber, V. (2018) Assessment of chronic stress in the horse with a focus on the ACTH-stimulation test. June 14 – 16, 2018; Forum of the American College of Veterinary Internal Medicine, Seattle, USA.
- (1) Sauer, F.J. (2017) Optimization of an ACTH challenge test to predict the risk of Equine Glandular Gastric Disease (EGGD) in horses. November 1-5, 2017; 10th Annual European College of Equine Internal Medicine Congress, Budapest, Hungary.
- (1 & 3) Ramseyer, A. and Sauer, F.J. (2018) Magengeschwüre und Stress-Test beim Sportpferd. January 15, 2018. Berner Fortbildungsabend, Avenches, Switzerland.
- (1 & 3) Lanz, S. and Sauer, F.J. (2018) Magengeschwüre – Was gibt es Neues?. November 10, 2018. Open-House Days – Henry Schein Animal Health, Switzerland.
- (3) Sauer, F.J. (2018) Untersuchungen zum Stress bei Hochleistungs- und Amateurpferden. April 19, 2018; 13. Jahrestagung Netzwerk Pferdeforschung, Avenches, Switzerland.
- (3) Sauer, F.J. (2019) Einfluss von Management- und Verhaltensfaktoren auf die Stress Reaktivität von Sportpferden. November 28 – 30, 2019; 51. Internationale Tagung Angewandte Ethologie, Freiburg, Germany.

Dissertations:

- (1) Sauer, F.J. (2018) Diagnostic accuracy of post-ACTH challenge salivary cortisol concentrations for identifying horses with equine glandular gastric disease. Dr.med.vet.; Vetsuisse Faculty, University Bern.
- (4) Kirchmaier, A. *In preparation.* Plasma steroid profiles before and after ACTH stimulation test in healthy horses. Dr.med.vet.; Vetsuisse Faculty, University Bern.

PhD:

- (2-5) Sauer, F.J. *In preparation.* Assessment of chronic stress in horses and development of a 'stress test' (adrenal cortisol response to ACTH) for stress-induced health problems. Dr.sc.nat.; Vetsuisse Faculty, University Bern.

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