

## Accepted Manuscript

Title: Fecal strongyle egg counts in horses with suspected pre-clinical pituitary pars intermedia dysfunction before and after treatment with pergolide

Authors: Garance Christen, Vinzenz Gerber, Johannes Hermanus van der Kolk, Caroline Franziska Frey, Nathalie Fouché



PII: S1090-0233(18)30088-1  
DOI: <https://doi.org/10.1016/j.tvjl.2018.03.007>  
Reference: YTVJL 5135

To appear in:

Accepted date: 21-3-2018

Please cite this article as: Garance Christen, Vinzenz Gerber, Johannes Hermanus van der Kolk, Caroline Franziska Frey, Nathalie Fouché, Fecal strongyle egg counts in horses with suspected pre-clinical pituitary pars intermedia dysfunction before and after treatment with pergolide (2018), <https://doi.org/10.1016/j.tvjl.2018.03.007>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

## Short Communication

### Fecal strongyle egg counts in horses with suspected pre-clinical pituitary pars intermedia dysfunction before and after treatment with pergolide

Garance Christen <sup>a,\*</sup>, Vinzenz Gerber <sup>a</sup>, Johannes Hermanus van der Kolk <sup>a</sup>, Caroline Franziska Frey <sup>b</sup>, Nathalie Fouché <sup>a</sup>

<sup>a</sup> *Swiss Institute of Equine Medicine, University of Bern and Agroscope, Länggassstrasse 124, CH- 3012 Bern, Switzerland*

<sup>b</sup> *Institute of Parasitology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, CH- 3012 Bern, Switzerland*

\* Corresponding author: Tel.: +41 31 631 22 43.

E-mail address: Garance.christen@vetsuisse.unibe.ch (G. Christen).

## Highlights

- Horses with pre-clinical pituitary pars intermedia dysfunction (PPID) did not have higher fecal egg counts (EPG) than healthy controls.
- In horses with pre-clinical PPID that received treatment with pergolide, EPG decreased over time compared to baseline EPG.
- There was no significant difference in EPG between pergolide and placebo treated animals.

## Abstract

Pituitary pars intermedia dysfunction (PPID) has been associated with diminished immune response in aged horses. This prospective study hypothesised that this may result in increased strongyle egg shedding in affected animals and that horses treated with pergolide would have reduced fecal egg counts (eggs per gram, EPG) compared to placebo-treated animals. Adrenocorticotrophic hormone (ACTH) concentrations and EPG were tested in 48 horses. There were no significant differences in baseline EPG between horses with pre-clinical PPID and healthy controls. There was no significant difference in EPG between horses with PPID after treatment with pergolide and placebo-treated animals. Using EPG as a marker of

immune function, these results did not support a proposed decrease in immune function in horses with pre-clinical PPID.

*Keywords:* Equine; Geriatric; Parasites; Placebo; PPID

Pituitary pars intermedia dysfunction (PPID) is a common disorder affecting about 20% of aged horses (McGowan et al., 2013b). It has been suggested that changes in circulating pituitary pars intermedia peptides are responsible for increased susceptibility to infection due to their role in immune function (McFarlane, 2014). These chronic effects on immune function could diminish defenses against intestinal parasites. We therefore hypothesized that horses with PPID would have higher fecal egg counts (eggs per gram, EPG) compared to healthy controls. Furthermore, we expected lower EPG in horses with pre-clinical PPID after 3 months of treatment with pergolide compared to placebo-treated animals.

In this prospective study, horses and ponies owned by a foundation for retired equids were examined clinically, and plasma ACTH and EPG were assessed. The study aimed to screen a population of aged horses for early onset of disease. None of the animals showed obvious clinical signs of PPID. The study was approved by the Animal Experimentation Committee of the Cantons of Jura and Berne, Switzerland (authorization number JU EXPE01; approval date 3 June 2014). The last anthelmintic treatment was administered at least 4 months prior to study commencement. The study period ranged from 1 July 2014 to 31 January 2015. Blood was sampled on two occasions: (1) all horses in July (ACTH<sub>1</sub>); and (2) in horses with pre-clinical PPID again in January (ACTH<sub>2</sub>). ACTH analysis was performed using a chemoluminescence immunoassay (Perkins et al., 2002). To exclude horses with equine metabolic syndrome, an oral sugar test was performed in July according to the protocol described by Schuver et al. (2014). For the purposes of the study, the PPID group included

horses with ACTH concentrations  $>35$  pg/mL in July<sup>1</sup>. The control group (CON) consisted of healthy horses with ACTH concentrations below the upper limit of the reference range. Horses in the PPID group were treated with 0.002 mg/kg BW pergolide (Prascend, Boehringer Ingelheim; PER) or placebo (PLA) orally for 3 months (1 October 2014 to 31 January 2015). Allocation to study group PER or PLA was determined by simple randomization using dedicated software<sup>2</sup>. Placebo treatment consisted of the same tablet as pergolide (Prascend, Boehringer Ingelheim) without the active ingredient.

Fecal samples were collected once daily during a 5-day period from freshly voided feces. A combined sedimentation-flotation method was performed using a previously published method (Boch and Bauer, 2006). Fecal egg counts were performed using the McMaster method (Kaufmann, 2013) on three occasions: (1) EPG-1 baseline: August; all horses; (2) EPG-2 baseline: September; all horses; and (3) EPG after treatment: January, PPID group treated with pergolide (PER) or placebo (PLA). For a hypothesised increase in EPG of approximately 100% in horses with PPID compared to healthy controls (McFarlane et al., 2010), a required sample size of 50 horses (25 horses with PPID and 25 control horses) was calculated<sup>3</sup>. Data was analysed using NCSS8 statistical software. *P*-values  $< 0.05$  were considered statistically significant. Comparisons of age distribution and EPG between groups (PPID vs. CON and PER vs. PLA) were performed using Kruskal-Wallis one-way ANOVA on ranks for non-parametric values. Correlations between ACTH<sub>1</sub>, EPG-1 and EPG-2 and between ACTH<sub>2</sub> and EPG-3 were calculated using linear regression. Baseline fecal egg counts (EPG-1 and EPG-2) were

---

<sup>1</sup> See: Durham, A.E., 2016. Seasonal Changes in ACTH Secretion. <http://liphookequinehospital.co.uk/wp-content/uploads/Seasonal-Changes-in-ACTH-Secretion2.pdf> (accessed 25 March 2018)

<sup>2</sup> See: Research Randomizer. [www.randomizer.org](http://www.randomizer.org) (accessed 25 March 2018).

<sup>3</sup> See: Sample Size Calculator. <http://clinicalcalc.com/stats/samplesize.aspx> (accessed 25 March 2018).

compared to EPG after treatment (EPG-3) in the PER and PLA groups using repeated measures analysis of variance.

Forty-eight horses with a mean  $\pm$  standard deviation (SD) age of  $24.8 \pm 3.6$  years (range, 14 to 32 years) were included in the study. Twenty-two were geldings and 26 were mares. ACTH<sub>1</sub> values ranged from 10.6 to 235 pg/mL (median 37.3 pg/mL; standard error [SE] 6.9). Twenty-four horses (50%) were diagnosed with PPID and 24 healthy horses (50%) were assigned to the CON group. Horses in the PPID group were significantly older than horses in the CON group ( $P < 0.01$ ; Table 1). Reported results for EPG included only strongyle eggs and are displayed in Table 1. In both groups, there was no significant difference between baseline EPG-1 ( $P = 0.66$ ) and baseline EPG-2 ( $P = 0.71$ ; Table 1). Plotting ACTH<sub>1</sub> concentrations against baseline EPG-1 and EPG-2 did not reveal statistically significant correlations ( $P = 0.26$  and  $P = 0.15$ , respectively; Figs. 1a, b). Of the 24 horses with pre-clinical PPID, five died of unrelated causes during the treatment period and were therefore excluded from the analysis for ACTH<sub>2</sub> and EPG-3. Ten horses in the PPID group were treated with pergolide and nine horses with placebo. ACTH<sub>2</sub> concentrations ranged between 7.4 and 479 pg/mL (median 45.4 pg/mL; SE 24.5). There was no significant difference in age ( $P = 0.05$ ) or ACTH<sub>1</sub> values ( $P = 0.7$ ) between the PER and PLA groups, but ACTH<sub>2</sub> values were significantly lower in the PER group ( $P < 0.01$ ; Table 1). There was no significant difference in EPG after treatment between the PER and PLA groups ( $P = 0.05$ ; Table 1). EPG-3 was significantly lower in the PER groups compared to baseline values ( $P = 0.03$ ), but not in the PLA groups ( $P = 0.88$ ). ACTH<sub>2</sub> concentrations were not significantly correlated with EPG-3 after treatment (Fig. 2).

Contrary to our hypothesis, horses with pre-clinical PPID did not have higher EPG than healthy control horses. In the PPID group, the difference in EPG after treatment in horses treated with pergolide compared with placebo-treated animals did not reach statistical

significance. There was a statistically significant difference between baseline EPG and EPG after treatment in horses treated with pergolide compared to placebo-treated animals. Whether this was due to the suspected influence of pergolide on the immune system, the gastrointestinal system of the horse, or direct dopaminergic effects on the parasite remains unclear. Direct effects on parasite burden would explain why differences did not seem to be associated with host disease status, but were associated with pergolide treatment, although this conclusion remains speculative at this stage. This part of the study was statistically underpowered and the results need to be confirmed with larger experimental groups, as well as ACTH and fecal testing at the same time of the year.

We defined the PPID group on the basis of ACTH concentrations rather than clinical signs, as none of the horses showed marked signs of advanced clinical disease. However, a reference standard method to diagnose PPID in cases with only subtle clinical signs and mildly elevated ACTH values remains unavailable. In future studies, additional ACTH measurements could be performed in autumn to increase the chances of correctly identifying affected animals (McGowan et al., 2013a). Our results differ from those of an earlier study where horses with PPID had higher EPG counts before and after anthelmintic treatment compared to healthy controls (McFarlane et al., 2010). The previous study included horses with more advanced disease, while our study investigated aged horses without obvious signs of PPID. Therefore, the discrepancy in study results might be based on the difference in the inclusion criteria.

Limitations of the present study include several important factors affecting parasite infection levels and thus EPG. Some of these factors may have been attenuated by standardised management protocols, while other factors, such as the immune status of the animal could not be addressed.

In our study, a proposed reduction in immune function in horses with pre-clinical PPID was not supported by increased fecal egg shedding by intestinal strongyles. Differences in EPG in horses with pre-clinical PPID after treatment with pergolide compared with placebo have not previously been reported.

#### **Conflict of interest statement**

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

#### **Acknowledgements**

The authors thank 'Die Stiftung für das Pferd' for access to the study population and good collaboration, Synlab for performing all blood analyses and Sylvie Froidevaux for help with the coprologic examinations.

## References

- Boch, J., Bauer, C., 2006. In: *Veterinärmedizinische Parasitologie*, sixth Edn. Paul Parey Verlag, Stuttgart, Germany, pp. 87-88.
- Kaufmann, J., 2013. In: *Parasitic infections of domestic animals: A diagnostic manual*, first Edn. Birkhäuser, Basel, Switzerland, pp. 7.
- McFarlane, D., 2014. Pathophysiology and clinical features of pituitary pars intermedia dysfunction. *Equine Veterinary Education* 26, 592-598.
- McFarlane, D., Hale, G.M., Johnson, E.M., Maxwell, L.K., 2010. Fecal egg counts after anthelmintic administration to aged horses and horses with pituitary pars intermedia dysfunction. *Journal of the American Veterinary Medical Association* 236, 330-334.
- McGowan, T.W., Pinchbeck, G.P., Mc Gowan, C.M., 2013a. Evaluation of basal plasma alpha-melanocyte-stimulating hormone and adrenocorticotrophic hormone concentrations for the diagnosis of pituitary pars intermedia dysfunction from a population of aged horses. *Equine Veterinary Journal* 45, 66-73.
- McGowan, T.W., Pinchbeck, G.P., McGowan, C.M., 2013b. Prevalence, risk factors and clinical signs predictive for equine pituitary pars intermedia dysfunction in aged horses. *Equine Veterinary Journal* 45, 74-79.
- Perkins, G.A., Lamb, S., Erb, H.N., Schanbacher, B., Nydam, D.V., Divers, T.J., 2002. Plasma adrenocorticotropin (ACTH) concentrations and clinical response in horses treated for equine Cushing's disease with cyproheptadine or pergolide. *Equine Veterinary Journal* 34, 679-685.



**Figure legends**

Fig. 1. Correlation of the concentration of adrenocorticotrophic hormone (ACTH) in July (ACTH<sub>1</sub>, pg/mL) and baseline fecal egg counts (eggs per gram feces, EPG) of all horses in August (Baseline EPG 1; a) and in September (Baseline EPG 2; b).

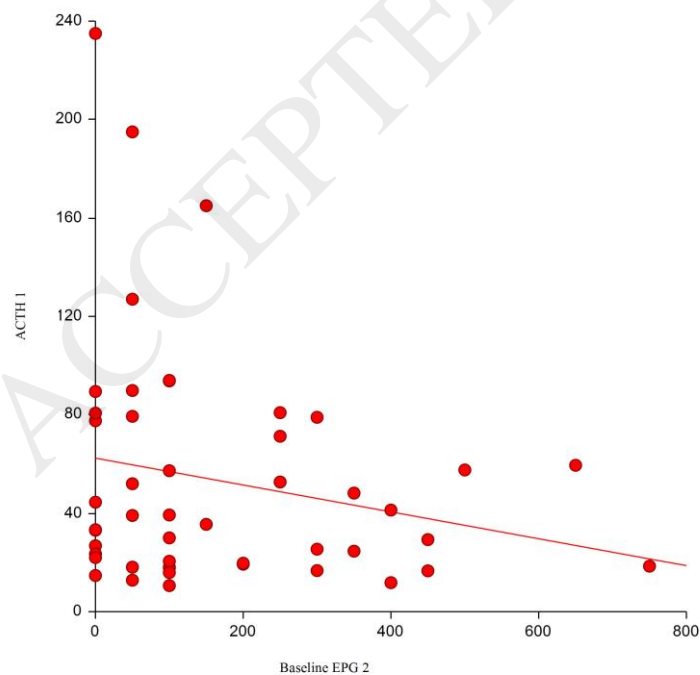
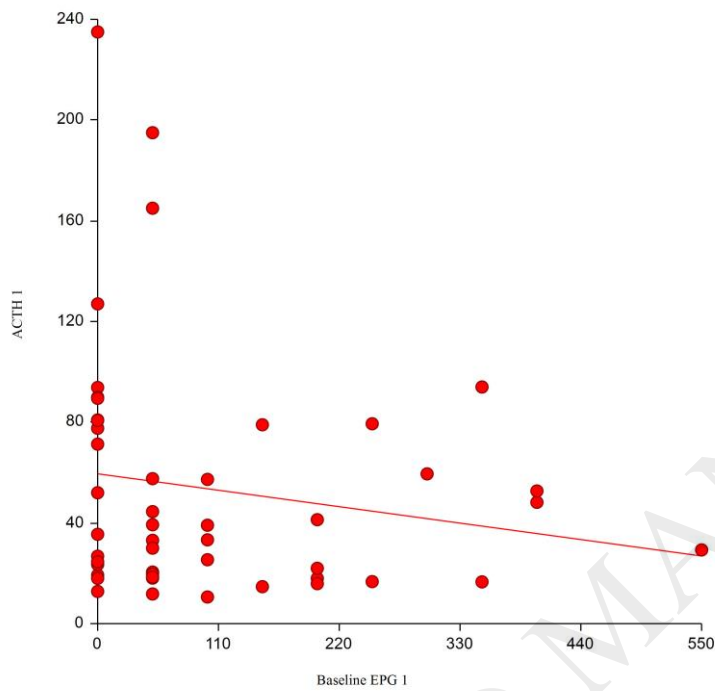
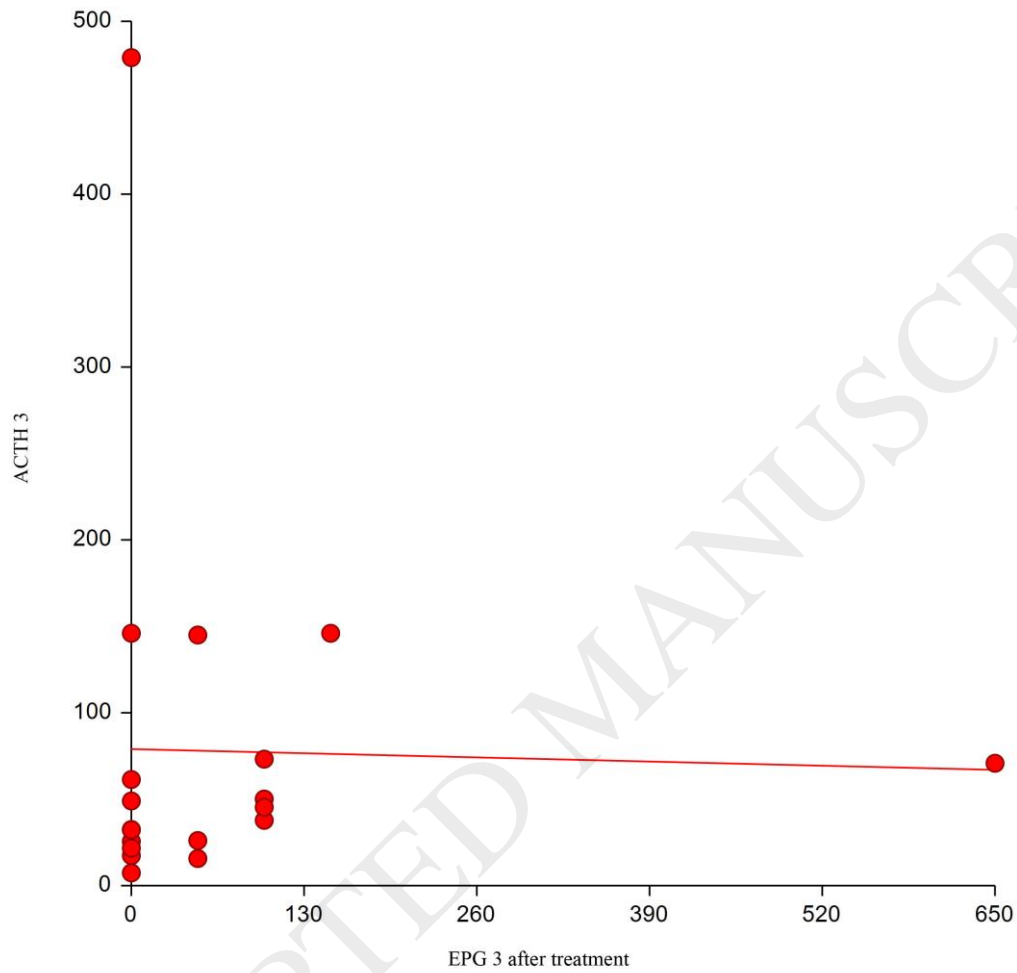


Fig. 2. Correlation of the concentration of adrenocorticotrophic hormone (ACTH) in January (ACTH<sub>2</sub>) and fecal egg counts (eggs per gram, EPG) after treatment (EPG 3 after treatment).



**Table 1**

Mean  $\pm$  standard deviation (SD) age, median (range) adrenocorticotrophic hormone (ACTH) concentrations (pg/mL) and median (range) fecal egg counts (egg per gram faeces, EPG) in horses with pituitary pars intermedia dysfunction (PPID,  $n=24$ ), control horses (CON,  $n=24$ ) and horses with PPID treated with pergolide (PER,  $n=10$ ) or with placebo (PLA,  $n=9$ ).

|  | PPID ( $n=24$ ) | CON ( $n=24$ )   | PER ( $n=10$ )  | PLA ( $n=9$ )   |
|--|-----------------|------------------|-----------------|-----------------|
| Age (years)                            | 26.7 $\pm$ 2.2  | 22.9 $\pm$ 3.7   | 27.4 $\pm$ 2.2  | 25.6 $\pm$ 1.8  |
| ACTH <sub>1</sub> (pg/mL) <sup>a</sup> | 78.3 (39.1-235) | 19.5 (10.6-35.5) | 75.2 (39.1-195) | 80.7 (39.3-127) |
| ACTH <sub>2</sub> (pg/mL) <sup>b</sup> | –               | –                | 25.9 (7.4-50.1) | 73.2 (37.8-479) |
| EPG-1 <sup>c</sup>                     | 50 (0-400)      | 50 (0-550)       | –               | –               |
| EPG-2 <sup>d</sup>                     | 100 (0-650)     | 100 (0-750)      | –               | –               |
| EPG-3 <sup>e</sup>                     | –               | –                | 0 (0-100)       | 100 (0-650)     |

<sup>a</sup> Baseline ACTH concentrations measured in July (all horses;  $n=48$ ).

<sup>b</sup> ACTH concentrations after 3 months of treatment with pergolide or placebo in January (horses in PER and PLA groups;  $n=19$ ). The number of horses in this category is  $n=19$  as five horses from group PPID died before blood samples for ACTH<sub>2</sub> were collected

<sup>c</sup> Baseline fecal egg counts in August.

<sup>d</sup> Baseline fecal egg counts in September.

<sup>e</sup> Fecal egg counts in January after 3 months of treatment with pergolide or placebo.