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# Association Between Adrenocorticotropic Hormone Concentration and Clinical Signs of Pituitary Pars Intermedia Dysfunction in Swiss and Austrian Equids

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## ABSTRACT

**Background:** There remains a scarcity of data concerning the relationship between the clinical signs associated with PPID and plasma ACTH concentrations.

**Objectives:** Report the frequency of clinical signs in the study cohort and identify individual clinical signs or combinations of clinical signs associated with high ACTH concentrations.

**Animals:** Two hundred eighty equids were examined by private veterinarians.

**Methods:** A cross-sectional study of animals with clinical suspicion of PPID was conducted between August and November. Private practitioners completed an online questionnaire reporting detailed information, including signalment, owner-reported history, and clinical observations during the examination. Plasma ACTH concentrations of each animal were measured. Associations between clinical signs and ACTH concentrations were examined.

**Results:** Age, month, and specific clinical signs (hair coat abnormalities, laminitis, and supraorbital fat) were univariably associated with high ACTH concentrations. The first three dimensions of the multiple correspondence analysis (MCA) described the aggregation of clinical signs according to a known semiological typicity. Dimensions 1, 2, and 3 corresponded to features indicative of equine metabolic syndrome, early-stage PPID, and advanced PPID, respectively. Further regression analysis showed that Dimensions 1 and 3, as well as age, were significant predictors of high ACTH levels.

**Conclusions and Clinical Importance:** The results indicate that particular clinical signs commonly associated with advanced stages of PPID are associated with high ACTH levels. Furthermore, in this data set, a phenotype compatible with metabolic syndrome was also linked to higher ACTH concentrations. These findings underscore the importance of seeking more sensitive biomarkers for the diagnosis of early PPID.

**Abbreviations:** ACTH, adrenocorticotropic hormone; EMS, equine metabolic syndrome; MCA, multiple correspondence analysis; PPID, pituitary pars intermedia dysfunction.

## 1 | Introduction

Pituitary pars intermedia dysfunction (PPID) is commonly diagnosed in older horses [1], and with the increasing population of aged equids, veterinarians are often tasked with diagnosing and treating this condition. Several studies have documented the prevalence of clinical signs in horses afflicted with PPID [1–16]. Among the existing literature, some studies described owner-reported histories of clinical signs [1, 15], while the majority rely on data collected by veterinarians [1, 2, 4–16]. Ideally, owner-reported observations and clinical evaluations conducted by veterinarians should be integrated since certain clinical signs, such as alterations in behavior require input from horse owners for accurate assessment. Conversely, veterinary assessments are indispensable as owners may misinterpret or misjudge the clinical manifestations of PPID. The most commonly reported clinical signs of PPID are abnormalities in hair coat condition and the occurrence of laminitis [17]. However, other more unspecific clinical signs such as weight loss, lethargy, or increased thirst and urination are reported inconsistently among studies [3, 5, 7–9, 12, 15, 16]. Signs that are considered "suggestive of PPID" or potential comorbidities [18], such as abnormal mammary secretions, are mentioned only sporadically. No studies to date have reported the frequency of suspensory ligament laxity in PPID-afflicted horses. While the published evidence offers valuable insights, to the authors knowledge no reports apart from the study by McGowan, Pinchbeck, and McGowan [1] have concomitantly investigated both owner observations and veterinary assessments, nor has the spectrum of potential clinical manifestations of PPID in horses and their correlation with adrenocorticotropic hormone (ACTH) levels been comprehensively explored.

This study aims to gain further insights into the relationship between clinical signs and ACTH values in horses suspected of suffering from PPID, based on a large data set. The first objective of the study was to report the frequency of clinical signs in the study cohort. The second objective was to identify individual clinical signs or combinations of clinical manifestations of PPID and their associations with elevated ACTH levels.

## 2 | Materials and Methods

This cross-sectional study was conducted between August and November 2022 in Austria (AT) and Switzerland (CH). Boehringer Ingelheim partnered with private veterinarians, incentivizing them with complimentary ACTH measurement vouchers. Veterinarians who had clinical suspicions of PPID in horses were given the opportunity to collect samples. Only animals older than 10 years not previously treated with pergolide were included in the study. The vouchers contained comprehensive guidelines for sample collection and shipment, ensuring prompt centrifugation of EDTA blood samples at low temperatures. After centrifugation, plasma was transferred to uncoated plastic tubes and either sent to the laboratory the same day or frozen until shipment. Samples reached the laboratory within 24 h. ACTH measurements were conducted using a chemiluminescence immunoassay in a commercial laboratory (IDEXX, Immulite 2000xpi). Additionally, the vouchers provided information to veterinarians about potential clinical signs and bloodwork alterations associated with PPID [18].

In addition, practitioners completed an online questionnaire, confirming owner consent for data utilization. The questionnaire gathered detailed information on the horse's signalment, owner-reported history, and clinical observations during the examination. This included sex (mare, stallion, and gelding), age, and breed classification based on primary categories in Switzerland and Austria. Four answer options were provided for clinical signs in the questionnaire: "yes," "possibly/slightly" (translated into "suspected" for the rest of this paper), "no," and "no answer possible." Veterinarians also had the option to manually enter other abnormalities into the questionnaire.

Data were analyzed using the statistical software NCSS[1] and R.[2] The missingness of our data set was explored with the R packages finalfit[3] and naniar.[4] List-wise deletion was employed because the analysis indicated missingness at random. Individuals with > 50% of missing values were excluded from the analysis (10 animals). Reported clinical signs with > 10% of missing values were also excluded (abnormal udder secretion in mares reported by owners and by clinicians, owner-reported fertility problems in mares, and clinician-reported altered menace response). An age-group variable was created with four categories to classify animals by age in years (< 16, 16–20, 21–25, and > 25), as well as a test-month variable with three categories to gather test results for the months in which the animals were sampled (August, September/October, and November). For ACTH concentration test results, a continuous variable was created by replacing the upper limit of test detection > 1250 ng/L with 1251 ng/L. Equids were also assigned to an ACTH_group based on their ACTH concentration (ACTH_low: ACTH concentration < 20 pg/mL in August, < 30 pg/mL in September and October and < 15 pg/mL in November; ACTH_equivocal: ACTH concentration ≥ 20 and ≤ 75 pg/mL in August, ≥ 30 and ≤ 90 pg/mL in September and October and ≥ 15 and ≤ 50 pg/mL in November; ACTH_high: ACTH concentration > 30 pg/mL in August, > 90 pg/mL in September and October and > 50 pg/mL in November). The reference values published by the Equine Endocrinology Group, which are normally used to diagnose clinical cases and should be interpreted in conjunction with clinical signs, were adopted for this study to categorize the ACTH groups [18].

Characteristics of the study cohort and reported clinical signs were summarized using descriptive statistics. The chi-squared test was used to compare the distribution of demographic variables between the two countries. Because ACTH values were not normally distributed, associations between ACTH concentration and other variables were assessed using Kruskal–Wallis one-way analysis of variance. Where relevant, the Dunn's post hoc test and Bonferroni adjustment for multiple testing were applied. The chi-squared test was also used to test the univariable association between ACTH_group (ACTH_low, ACTH_equivocal, and ACTH_high) and the other variables.

To examine potential relationships between the reported clinical signs, a multiple correspondence analysis (MCA) was run on the data set with the R packages FactoMineR[5] and Factoshiny.[6] The variables ACTH_group and age_group were selected as illustrative (Supporting Information) variables whereas the clinical signs were set as active components (used to build the MCA dimensions). While running the MCA, remaining missing values of the data set were automatically imputed with a two-dimensional MCA model and nine remaining outliers were suppressed because of their strong contribution to the construction of the plane (cumulative contribution = 10.9%). The relevant dimensions (synthetic variables) generated by the MCA were selected to summarize the original variables linked to reported clinical signs and used in a multinomial logistic regression to assess their propensity to predict ACTH_group, taking into account the age category as a confounding factor. The Akaike Information Criteria was used to select the model with the best fit to our data.

## 3 | Results

A total of 280 equids (hereinafter referred to as horses) were enrolled in the study, ranging in age from 11 to 35 years, with a mean age of 20.9 years ± standard deviation (SD) of 5.3. The characteristics of the study cohort are summarized in Table 1. The sexes were evenly distributed between females and males (including gelding and stallions). The most common breeds were Warmblood type, Pony types, Freiberger, Icelandic Horses, and Haflinger. The cohort characteristics between the two countries were comparable.

Table 1 (and Supporting Information 1) presents the ACTH concentrations in relation to the month at the time of sampling and sex, age, and breed of the horses. Overall, ACTH concentrations

**TABLE 1** | Study cohort characteristics (absolute numbers [*n*], proportions compared to the total population [%]) and measured ACTH concentration (ng/L) according to season, sex, age group, and breed.

| | Total | | ACTH | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***n*** | **%** | **Mean** | **SD** | **Median** | **Q1** | **Q3** | ***p*** | |
| Horses | 280 | | 189.4 | 281.6 | 68.7 | 38.0 | 203.3 | | |
| Months | | | | | | | | | |
| August | 38 | 13.6 | 187.9 | 330.7 | 60.4 | 34.4 | 185.5 | | |
| September/October | 222 | 79.3 | 196.2 | 274.8 | 73.6 | 42.4 | 223.5 | | |
| November | 20 | 7.1 | 117.2 | 258.6 | 34.6 | 25.1 | 82.8 | **0.007 | vs. Sept/Oct |
| Sex | | | | | | | | | |
| Female | 135 | 48.2 | 207.6 | 294.5 | 68.8 | 39.6 | 221.0 | | |
| Male | 144 | 51.4 | 173.4 | 269.6 | 69.0 | 38.0 | 183.3 | | |
| Unknown | 1 | 0.4 | | | | | | | |
| Age | | | | | | | | | |
| < 16 | 54 | 19.3 | 116.2 | 229.1 | 41.7 | 27.5 | 69.3 | ***< 0.001 | vs. 21–25 and > 25 |
| 16–20 | 78 | 27.9 | 153.2 | 283.6 | 55.6 | 33.7 | 110.0 | **< 0.01 | vs. 21–25 and > 25 |
| 21–25 | 91 | 32.5 | 200.6 | 247.8 | 103.0 | 50.4 | 255.0 | | |
| > 25 | 56 | 20.0 | 294.8 | 345.8 | 126.0 | 59.3 | 407.8 | | |
| Unknown | 1 | 0.4 | | | | | | | |
| Mean (SD) | 20.9 | 5.3 | | | | | | | |
| Breed and breed type | | | | | | | | | |
| Warmblood | 96 | 34.3 | 181.6 | 269.3 | 65.0 | 31.9 | 215.0 | | |
| Pony | 66 | 23.6 | 289.6 | 389.8 | 86.9 | 47.7 | 293.8 | | |
| Freiberger | 30 | 10.7 | 115.2 | 144.2 | 56.6 | 43.7 | 82.3 | | |
| Icelandic | 26 | 9.3 | 163.3 | 227.6 | 68.5 | 37.1 | 196.0 | | |
| Haflinger | 20 | 7.1 | 184.2 | 283.6 | 72.7 | 45.4 | 160.5 | | |
| Baroque | 15 | 5.4 | 98.6 | 97.6 | 49.5 | 36.8 | 148.5 | | |
| Purebred | 11 | 3.9 | 154.1 | 180.6 | 82.7 | 48.7 | 174.5 | | |
| Quarter horse | 8 | 2.9 | 163.5 | 219.4 | 64.7 | 40.6 | 162.3 | | |
| Other | 8 | 2.9 | 76.6 | 76.0 | 50.8 | 24.2 | 104.8 | | |

*Note:* Kruskal–Wallis test (** < 0.01, *** < 0.001) and Bonferroni post hoc test (values) significance results are shown.

ranged from 8.2 to 1251 ng/L, with a median of 68.7. Horses sampled in September/October exhibited higher ACTH concentrations than those sampled in August and November, with a significant difference noted between September/October and November. No difference in ACTH concentrations was observed between females and males. Horses in the older age groups demonstrated higher ACTH concentrations than those in the younger age groups. When horses were classified into groups according to their ACTH concentration, 118 (42.1%) cases were classified in group ACTH_high, 131 (46.8%) in group ACTH_equivocal, and 31 (11.1%) in group ACTH_low. A significant increase of horses classified in group ACTH_high was observed with advancing age ($p < 0.001$).

Table 2 presents an overview of the frequency of clinical signs reported by owners (anamnesis) and observed by veterinarians during the clinical examination. The historical clinical signs most frequently reported in the whole study cohort were delayed shedding, followed by weight loss, poor performance, lethargy, and laminitis. Based on their clinical examination, veterinarians most frequently reported signs of regional adiposity, muscle atrophy, hypertrichosis, and acute laminitis.

Comparing ACTH concentrations according to the expression of specific clinical signs (see Table 3), horses with a history of delayed shedding exhibited significantly higher ACTH test results than those where this clinical sign was suspected or absent. Similarly, horses with a history of laminitis demonstrated significantly higher ACTH test results than those without this clinical sign. Horses displaying clinical hypertrichosis exhibited significantly higher ACTH values than horses without this clinical sign or those classified as suspected. Higher ACTH values were also observed in horses with acute and chronic laminitis. Furthermore, horses with present or suspected supraorbital fat pads showed higher ACTH values compared to horses without them, although this difference was only significant for horses classified as suspected.

The first three dimensions generated by MCA, collectively expressing 21.4% of the total inertia (variability of the data set), were retained (see Supporting Information 2). Additionally, Supporting Information 2 displays the cloud of individuals in two planes (Dimension 1, Dimension 2) and (Dimension 1, Dimension 3). The clouds appear relatively homogeneous and well dispersed. Upon visual inspection, horses that are negatively correlated with Dimensions 1 and 3 are less likely to have ACTH concentrations above the seasonally adjusted reference range. However, there is no evident visual clustering of individuals overall.

The clinical signs, most closely correlated visually (and mathematically) with the three dimensions are described in Figure 1. Dimension 1 grouped individuals characterized by a positive coordinate on the axis and sharing high frequency for the clinical signs: regional adiposity, supraorbital fat pads, history of laminitis, chronic laminitis, and increased appetite. Horses with a positive coordinate on the Dimension 2 axis shared high frequency for clinical signs: poor performance, weight loss, muscle atrophy, lethargy, and acute laminitis. The axis of Dimension 3 is positively correlated with individuals sharing a high frequency for muscle atrophy, delayed shedding, weight loss, and hypertrichosis.

**TABLE 2** | Frequency of clinical signs reported by owners (anamnesis) and veterinarians (clinical examination) in decreasing order across the study population ($n = 280$).

| | Yes | | Suspected | |
|---|---|---|---|---|
| | ***n*** | **%** | ***n*** | **%** |
| Anamnesis | | | | |
| Delayed shedding | 110 | 39.3 | 77 | 27.5 |
| Weight loss/loss of condition | 73 | 26.1 | 45 | 16.1 |
| Poor/reduced performance | 68 | 24.3 | 59 | 21.1 |
| Lethargy/apathy | 66 | 23.6 | 54 | 19.3 |
| History of laminitis | 62 | 22.1 | 9 | 3.2 |
| Recurrent or chronic infections | 43 | 15.4 | 27 | 9.6 |
| Increased/excessive appetite | 36 | 12.9 | 29 | 10.4 |
| Increased drinking/excessively wet bedding | 30 | 10.7 | 22 | 7.9 |
| Excessive sweating | 27 | 9.6 | 33 | 11.8 |
| Neurological signs | 10 | 3.6 | 10 | 3.6 |
| Absent/decreased sweating | 0 | 0.0 | 5 | 1.8 |
| Clinical examination | | | | |
| Other signs of regional adiposity | 73 | 26.1 | 45 | 16.1 |
| Muscle atrophy | 67 | 23.9 | 77 | 27.5 |
| Hypertrichosis | 55 | 19.6 | 79 | 28.2 |
| Acute laminitis | 52 | 18.6 | 10 | 3.6 |
| Supraorbital fat pads | 45 | 16.1 | 43 | 15.4 |
| Chronic laminitis | 35 | 12.5 | 28 | 10.0 |
| Pastern dermatitis | 16 | 5.7 | 18 | 6.4 |
| Suspensory ligament laxity, hind legs | 13 | 4.6 | 14 | 5.0 |
| Chronic corneal ulcers/keratitis/conjunctivitis | 11 | 3.9 | 7 | 2.5 |
| Suspensory ligament laxity, front legs | 9 | 3.2 | 13 | 4.6 |

Finally, when assessing the properties of the synthetic variables (dimensions) generated by the MCA to predict the assignment of the horses to group ACTH_high, the best-fitted model of multinomial regression was the one with Dimensions 1 and 3 as predictors and age group as a confounding factor

**TABLE 3** | Comparative ACTH concentrations (ng/L) according to the expression of certain clinical signs.

| | Mean | SD | Median | Q1 | Q3 | *p* | |
|---|---|---|---|---|---|---|---|
| Anamnesis | | | | | | | |
| Delayed shedding | | | | | | | |
| No | 132.1 | 216.5 | 51.8 | 36.2 | 110.0 | | |
| Suspected | 135.9 | 207.2 | 62.9 | 32.8 | 120.0 | | |
| Yes | 265.9 | 337.4 | 119.0 | 50.0 | 320.5 | ***<0.001 | vs. no and suspected |
| Laminitis | | | | | | | |
| No | 159.7 | 247.2 | 58.7 | 34.8 | 184.5 | | |
| Suspected | 151.7 | 218.7 | 53.0 | 45.7 | 81.5 | | |
| Yes | 288.5 | 363.7 | 105.5 | 57.3 | 378.8 | **0.001 | vs. no |
| Clinical examination | | | | | | | |
| Hypertrichosis | | | | | | | |
| No | 134.0 | 227.4 | 51.2 | 35.2 | 104.5 | | |
| Suspected | 148.3 | 189.7 | 80.9 | 36.9 | 194.0 | | |
| Yes | 397.8 | 410.4 | 233.0 | 72.7 | 647.5 | ***<0.001 | vs. no and suspected |
| Acute laminitis | | | | | | | |
| No | 163.0 | 252.6 | 64.0 | 36.1 | 182.0 | | |
| Suspected | 114.2 | 110.4 | 68.1 | 51.7 | 151.8 | | |
| Yes | 316.6 | 374.9 | 126.5 | 49.4 | 455.8 | **0.005 | vs. no |
| Chronic laminitis | | | | | | | |
| No | 172.8 | 261.5 | 65.6 | 35.6 | 192.3 | | |
| Suspected | 231.7 | 344.1 | 69.4 | 43.9 | 291.3 | | |
| Yes | 269.9 | 341.0 | 110.0 | 60.6 | 390.5 | *0.031 | vs. no |
| Supraorbital fat pads | | | | | | | |
| No | 159.3 | 253.8 | 61.2 | 36.3 | 169.5 | | |
| Suspected | 270.1 | 347.5 | 112.0 | 54.9 | 319.5 | *0.018 | vs. no |
| Yes | 232.4 | 315.4 | 82.8 | 43.2 | 237.0 | | |

*Note:* Kruskal–Wallis test (** < 0.01, *** < 0.001) and Bonferroni post hoc test (values) significance results are shown.

(see Supporting Information 2). Dimension 1 was a significant predictor of ACTH_high (OR = 8.87, $p = 0.003$) and Dimension 3 was a significant predictor of both ACTH_equivocal and ACTH_high (OR = 5.18, $p = 0.018$ and OR = 10.77, $p = 0.001$, respectively). Aging was found to be a significant confounding factor in predicting ACTH_high (vs. group < 16 years, groups 16–20: OR = 4.02, $p = 0.021$, 21–25: OR = 14.89, $p < 0.001$, > 25: OR = 11.77, $p < 0.001$; see Supporting Information 2).

## 4 | Discussion

This research provides insights into the relationship between clinical signs in horses suspected of having PPID by private practitioners, and ACTH concentrations. Furthermore, using MCA, clusters of clinical signs were identified in this cohort of horses, and associations between these clusters and ACTH levels were explored. In the univariable analysis, hair coat abnormalities and laminitis as well as supraorbital fat deposits were associated with high ACTH concentrations. Using MCA, we identified three distinct groups of horses, but only the clinical signs associated with horses in Dimensions 1 and 3 were found to be associated with high ACTH concentrations. Horses in Dimension 1 showed signs compatible with equine metabolic syndrome, while those in Dimension 3 exhibited signs of advanced clinical disease of PPID. These results reinforce the idea that ACTH is not a sensitive biomarker for diagnosing PPID, especially in the early stages of this disorder [19].

The study cohort primarily consisted of older horses, reflecting a typical group of horses affected with PPID [20]. In this study cohort, the age of the horses significantly influenced the ACTH results, with older horses displaying higher values, consistent with findings previously documented in the literature [1, 21]. Indeed,

$`Dim 1`

| | R2 | p.value |
|---|---|---|
| C-regional adiposity | 0.55 | 0.00 |
| C-supraorbital fat | 0.51 | 0.00 |
| A-laminitis | 0.48 | 0.00 |
| C-chronic laminitis | 0.48 | 0.00 |
| A-increased appetite | 0.27 | 0.00 |
| A-increased sweating | 0.16 | 0.00 |
| A-lethargy | 0.15 | 0.00 |
| C-acute laminitis | 0.13 | 0.00 |
| C-suspensory front | 0.12 | 0.00 |
| C-hypertrichosis | 0.10 | 0.00 |
| A-poor performance | 0.10 | 0.00 |
| A-infections | 0.10 | 0.00 |
| C-suspensory hind | 0.09 | 0.00 |
| A-weight loss | 0.08 | 0.00 |
| C-muscle atrophy | 0.05 | 0.00 |
| A-PU/PD | 0.04 | 0.01 |
| Age-group | 0.04 | 0.02 |

$`Dim 2`

| | R2 | p.value |
|---|---|---|
| A-poor performance | 0.43 | 0.00 |
| A-weight loss | 0.36 | 0.00 |
| C-muscle atrophy | 0.32 | 0.00 |
| A-lethargy | 0.26 | 0.00 |
| C-acute laminitis | 0.23 | 0.00 |
| C-hypertrichosis | 0.21 | 0.00 |
| C-pastern dermatitis | 0.16 | 0.00 |
| A-infections | 0.14 | 0.00 |
| C-suspensory front | 0.13 | 0.00 |
| A-delayed shedding | 0.10 | 0.00 |
| C-suspensory hind | 0.09 | 0.00 |
| A-laminitis | 0.09 | 0.00 |
| A-increased sweating | 0.08 | 0.00 |
| A-neurological signs | 0.08 | 0.00 |
| A-PU/PD | 0.08 | 0.00 |
| C-chronic laminitis | 0.08 | 0.00 |
| C-supraorbital fat | 0.05 | 0.00 |
| A-decreased sweating | 0.03 | 0.01 |

**FIGURE 1** | Description of the MCA three first dimensions. The link between the dimension and clinical signs is shown (results from 1-way ANOVA). Images visualize the horse type described by each dimension. Figure legend: A = Clinical signs reported by the owners during the anamnesis; C = Clinical signs identified during the veterinary examination.

age was more strongly associated with autumnal ACTH concentrations than any combination of clinical signs. It remains unclear whether this is due to a higher prevalence of PPID in older horses or reflects a physiological rise in ACTH levels with age.

September and October exhibited the highest ACTH results in comparison to August and November, consistent with findings from previous studies [22, 23]. Also, in accordance with the previous reports, we found no difference in the ACTH measurements between females and males [1, 11, 17]. One previous study revealed that Arabian horses, donkeys, Shetland ponies, and Welsh breeds exhibited notably elevated ACTH concentrations compared to other breeds during the autumn months [23], and higher ACTH results in ponies compared to Thoroughbred horses were confirmed in a second study examining healthy individuals [24]. No breed differences were identified in this cohort of animals.

Among the clinical signs reported by owners and veterinarians, hair coat abnormalities and laminitis were associated with significantly higher ACTH results. ACTH concentrations were also higher in horses with supraorbital fat deposits compared to horses that did not display this clinical sign. However, only the comparison between "suspected" and "no" supraorbital fat deposits reached statistical significance. In this study, we were only able to identify an association between these clinical signs and ACTH levels. As ACTH is thought to be partly biologically inactive in horses with PPID [25], it is quite possible that there is no direct causal relationship between ACTH and the clinical signs observed.

The MCA provided valuable insights into the relationships between clinical signs and ACTH concentrations in horses. To perform the MCA, it was necessary to categorize the ACTH values. We decided to use the thresholds provided by the Equine Endocrinology Group [18] for the diagnosis and interpretation of ACTH levels in clinical cases. Even though ACTH values should always be interpreted in a clinical context, for the purpose of this categorization, these published thresholds offered the most

$`Dim 3`

| | R2 | p.value |
|---|---|---|
| C-muscle atrophy | 0.31 | 0.00 |
| A-delayed shedding | 0.30 | 0.00 |
| A-weight loss | 0.29 | 0.00 |
| C-hypertrichosis | 0.23 | 0.00 |
| A-poor performance | 0.20 | 0.00 |
| C-suspensory front | 0.16 | 0.00 |
| A-lethargy | 0.14 | 0.00 |
| C-regional adiposity | 0.14 | 0.00 |
| C-suspensory hind | 0.13 | 0.00 |
| C-chronic laminitis | 0.12 | 0.00 |
| C-acute laminitis | 0.09 | 0.00 |
| A-infections | 0.07 | 0.00 |
| ACTH_groups | 0.05 | 0.00 |
| C-pastern dermatitis | 0.06 | 0.00 |
| A-increased appetite | 0.06 | 0.00 |
| A-laminitis | 0.06 | 0.00 |
| C-supraorbital fat | 0.04 | 0.01 |
| Age-group | 0.04 | 0.02 |
| A-increased sweating | 0.04 | 0.02 |

**FIGURE 1** | Continued.

appropriate means of classification. Some groups of clinical signs often occur together and Dimensions 1, 2, and 3 combined these characteristics as illustrated in Figure 1. Dimension 1 grouped individuals with a high frequency of regional adiposity (including supraorbital fat deposits), acute and chronic laminitis and increased appetite. Dimension 2 was associated with poor performance, weight loss, muscle atrophy and a change in the horse's attitude, such as lethargy as well as acute laminitis. Dimension 3 was associated with delayed shedding and hypertrichosis, muscle atrophy and weight loss. Dimensions 1 and 3 were associated with high ACTH concentrations, with Dimension 3 showing the strongest association with the groups ACTH_high and ACTH_equivocal. It should be noted though, that age group was a stronger predictor of ACTH_group than any combination of clinical signs in these horses. From a clinical perspective, equine metabolic syndrome would be a significant differential diagnosis in animals fitting Dimension 1 and it is therefore not clear why the clustering of these clinical signs was associated with high ACTH concentrations in this cohort of animals. One possible explanation is that ponies exhibit higher ACTH levels in autumn compared to other breeds [23]. However, the same reference values were uniformly applied for categorizing into ACTH_groups across all breeds. Consequently, it is plausible that an overrepresentation of ponies in Dimension 1 could skew the association with a higher risk of PPID. While we lacked statistical evidence of a breed effect, such an association cannot be entirely discounted. The MCA was therefore repeated excluding the ponies (data not shown). Dimensions 1 and 2 still grouped the same clinical signs, while in Dimension 3, clinical hypertrichosis was replaced in the ranking by a history of poor performance and suspensory laxity on both front and hind limbs, when ponies were excluded. Dimension 1 and age_group remained predictive for ACTH-group in the regression analysis, but Dimension 3 was no longer associated with high ACTH concentrations, highlighting the strong association between hypertrichosis and ACTH levels.

Clinical signs from Dimension 2 were not associated with an increasing ACTH concentration. Possibly, these horses were in an early stage of PPID that is not yet associated with elevated ACTH levels. Classical signs of PPID, such as delayed shedding, hypertrichosis, and laminitis, remain the most reliable predictors of PPID in horses, as previously noted [26], when PPID diagnosis is based on ACTH concentrations. This result also suggests that ACTH is particularly suitable as a biomarker for late-phase PPID, as coat changes and laminitis usually occur at an advanced stage. It emphasizes the importance of focusing further research on finding biomarkers that detect PPID at an earlier stage.

The study was conducted during the autumn months, and it is unclear whether the results would be reproducible in other seasons. Certain clinical signs, such as coat changes, and ACTH levels [22], as well as metabolic activity [27, 28], vary depending on the time of year. Nonetheless, the study was carried out during a season when ACTH measurement is considered more sensitive than at other times of the year.

One limitation of this study is that data were prospectively collected from a convenience sample of horses, specifically those suspected of having PPID by their attending veterinarian. This is particularly pertinent when considering the frequency of clinical signs across the entire study cohort. Furthermore, the examinations were conducted by various veterinarians who likely possessed differing levels of familiarity with PPID. Blood sampling was also performed by private veterinarians, and the possibility cannot be discounted that pre-analytical factors such as specific EDTA tubes and the storage or shipping conditions of blood samples might have influenced ACTH values. To mitigate both effects, veterinarians were provided with a list of potential clinical signs associated with PPID and instructions for sample processing along with the voucher. Further influencing factors that could have affected ACTH levels, such as pain or training, cannot be ruled out with the present study design. Research on PPID generally faces limitations due to the absence of a definitive gold standard diagnostic test other than postmortem examinations of the pituitary gland. Clinically, it is recommended that ACTH measurements be interpreted within the broader clinical context rather than used as a standalone screening test [19]. Determining the true value of ACTH as a biomarker for PPID is challenging, as ACTH levels are concurrently used to classify horses as PPID-positive. This circular approach complicates the objective validation of ACTH's diagnostic utility.

In conclusion, this study enhances our understanding of PPID and emphasizes the importance of considering age, and specific clinical signs in the diagnosis of this condition. Within this study, a clear correlation was observed between specific clinical signs associated with PPID and elevated ACTH levels. Particularly notable were alterations in coat shedding, the occurrence of laminitis, weight loss, and muscle atrophy.


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#### Disclosure

Authors declare no off-label use of antimicrobials.

#### Ethics Statement

Authors declare no Institutional Animal Care and Use Committee (IACUC) or other approval was needed as data were analyzed from horses who received an ACTH measurement as part of their clinical examination. Authors declare human ethics approval was not needed.

#### Conflicts of Interest

Alexandre Scherer and Barbara Freudenschuss were at the time of project implementation employees of Boehringer Ingelheim. Nathalie Fouché, Vinzenz Gerber, Camille Doras, and Gertraud Schüpbach received partial funding by Boehringer Ingelheim for study design, statistical analysis, and manuscript preparation.

#### Endnotes

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**Supporting Information**

Additional supporting information can be found online in the Supporting Information section.