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Factors associated with survival, laminitis and insulin dysregulation in horses diagnosed with equine pituitary pars intermedia dysfunction

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Summary

Background: Pituitary pars intermedia dysfunction (PPID) is a commonly described endocrine disorder in higher latitudes of the Northern hemisphere but the description of the disease at lower latitudes and in the Southern hemisphere is limited.

Objectives: Document the clinical features of PPID at different Australian latitudes and climates, and investigate factors associated with survival, laminitis and insulin dysregulation (ID).

Study design: Retrospective study of 274 equids from 8 institutions across Australia.

Methods: A diagnosis of PPID was based on endogenous ACTH, overnight dexamethasone suppression test, thyrotropin-releasing hormone stimulation test or necropsy. Clinical and clinicopathologic characteristics of PPID and therapeutic responses were investigated. Laminitis was diagnosed by radiographic or histologic changes and ID was diagnosed based on endogenous insulin, an oral glucose test or a 2-step insulin-response test.

Results: Being a pony, having a higher body condition score and pergolide administration were associated with survival. The clinical presentation of PPID changed with latitude and climate, with anhidrosis and polyuria/polydipsia more commonly recognised at lower latitudes. Laminitis was diagnosed in 89.9% of cases and ID was present in 76.5% of cases in which they were investigated.

Main limitations: Despite the sample size, the lack of uniform testing at all locations (primary or referral cases) and in the incompleteness of datasets limited the power of the statistical analyses.

Conclusions: PPID can present with variable signs at different latitudes and climates, and ID should be investigated in equids diagnosed with PPID. Adequate body condition and administration of pergolide are fundamental in PPID management.

Abbreviations

α-MSH	α-Melanocyte-stimulating hormone
ACTH	Adrenocorticotropic hormone

CLIP	Corticotropin-like intermediate lobe peptide
EMS	Equine Metabolic Syndrome
ID	Insulin dysregulation
ODST	Overnight dexamethasone suppression test
OGT	Oral glucose test
POMC	Proopiomelanocortin
PPID	Pituitary pars intermedia dysfunction
QLD	Queensland
SA	South Australia
TAS	Tasmania
TRH	Thyrotropin-releasing hormone
VIC	Victoria
WA	Western Australia

Introduction

Pituitary pars intermedia dysfunction (PPID) is a progressive disorder of aged equids [1]. This disease is common and reported in over 20% of horses older than 15 years of age [2]. Degeneration of hypothalamic dopaminergic neurons is thought to result in a loss of tonic inhibition of the melanotropes located in the pars intermedia of the pituitary gland [3]. In the absence of inhibition, the melanotrope activity increases resulting in adenoma formation and dysregulated proopiomelanocortin (POMC) secretion [1]. After secretion, POMC is cleaved into adrenocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH), β-endorphin, corticotropin-like intermediate lobe peptide (CLIP) and other peptides. Although dysregulated POMC secretion results in increased ACTH and α-MSH concentrations, the exact consequences of increased concentrations of those hormones are poorly understood [4-6].

At high northern latitudes, the clinical features of PPID have been extensively described and clinical signs include hypertrichosis, laminitis, hyperhidrosis and opportunistic infections [1]. Although the clinical signs of PPID at an advanced stage are easy to recognise, diagnosis of subclinical PPID is more problematic as age-related changes can mask subtle manifestations [2]. Several tests have been developed to diagnose PPID but endogenous ACTH is the most commonly used [7-9]. Plasma concentrations of ACTH change with season and geographical location, as daylight duration and climate have been shown to play a major role on pituitary gland activity [5; 10-14]. Although a direct link between POMC-derived peptide

concentrations and clinical signs has not been elucidated, some reports suggest that PPID could have different presentations in different climatic zones [15]. In tropical climates, signs such as anhidrosis and heat stress have been reported, suggesting that, in certain regions, PPID may not present as classically described and that milder PPID cases could be missed [15].

Medical therapy with pergolide is the mainstay of PPID treatment [16]. However, up to 73% of PPID cases are euthanised for PPID-related disorders and euthanasia of 50% of cases has been reported within 4.5 years of diagnosis [10; 17]. One of the most debilitating complications of PPID is laminitis and one-third of PPID cases have been diagnosed with concurrent equine metabolic syndrome (EMS) and insulin dysregulation (ID) [18]. PPID and EMS are distinct conditions, and although PPID does not necessarily interfere with insulin regulation, the presence of ID in PPID cases is associated with laminitis [18; 19].

Our study aimed at providing accurate documentation of the clinical features of PPID at lower southern latitudes and at investigating the factors associated with survival, laminitis and ID in equids diagnosed with PPID.

Materials and Methods

Data collection

Medical records from 8 Australian institutions covering Queensland (QLD), Victoria (VIC), South Australia (SA), Western Australia (WA) and Tasmania (TAS) over a 15-year period were reviewed. Postal codes of individual cases were used to determine latitudes and absolute values were considered.

A diagnosis of PPID was based on the results of an endogenous ACTH, overnight dexamethasone suppression test (ODST), thyrotropin-releasing hormone (TRH) stimulation test or necropsy [1]. For endogenous ACTH, cut-off values were, for autumn (or dynamic phase) 101 pg/mL in northern QLD, 94 pg/mL in southern WA, 75 pg/mL in TAS and 77.4 pg/mL in southern SA, VIC and southern QLD, and, for other seasons (or quiescent phase), 67 pg/mL in northern QLD, 43 pg/mL in southern WA, 46 pg/mL in TAS and 29.7 pg/mL in southern SA, VIC and southern QLD [14; 20]. For the ODST, cortisol concentrations above 1 µg/dL (27.8 nmol/L) 15 hours after intramuscular injection of 40 µg/kg of dexamethasone was considered positive for PPID [21]. For the TRH stimulation test, ACTH above 110 pg/mL or 65 pg/mL, 10 or 30 minutes respectively after intravenous injection of 1 mg of TRH was considered positive for PPID [22]. Cases where diagnosis was solely based on clinical signs were excluded.

Data collected included signalment, date, season and climate at month of diagnosis (average rainfalls, average ambient temperatures, average humidity and average daylength), complaint, owner-reported clinical signs, history of previous disorders, referral, physical examination and bloodwork on presentation, method of diagnosis, investigation of laminitis, investigation of ID, treatment prescribed, follow-up and survival (follow-up and alive at last recheck). Insulin dysregulation was diagnosed with a 2-step insulin-response test (<50%-decrease in blood glucose after 0.1 IU/kg of intravenous insulin), an oral glucose test (OGT, serum insulin >85 µIU/mL after an oral glucose challenge) or an endogenous fasted insulin concentration >20 µU/mL [18; 23-25]. Laminitis was diagnosed based on radiographic evidence or at necropsy [26]. Insulin and ACTH were measured using a chemiluminescent assay at all institutions except one, where a radioimmunoassay was used.

Data analysis

Horses were grouped by outcome of interest (survival, laminitis and ID) and compared, with $p<0.05$ considered significant. Normality of continuous data was assessed with a Shapiro-Wilk test. Normally distributed data were reported as mean ± s.d. and compared using unpaired *t*-tests whereas non-normally distributed data were reported as median [range] and compared using Mann–Whitney *U*-tests. Categorical data were compared using a Chi-square test or a Fisher's exact test depending on expected counts. The associations between clinical signs, clinicopathologic factors and treatment with the outcomes of interest were investigated using backward stepwise logistic regression with $p<0.2$ to enter and $p<0.05$ to remain in the model. The final logistic regression model fit was evaluated using the Hosmer–Lemeshow Goodness-of-Fit test. Statistical analysis was performed using commercially available statistical software[a,b].

Results

Signalment and history

Two hundred and seventy-four cases met the inclusion criteria: 121 (44.2%) from VIC, 65 cases (23.7%) from QLD, 43 (15.7%) from TAS, 30 (10.9%) from SA and 15 (5.5%) from WA (Fig 1). One hundred and fifty-six cases (56.9%) were primary cases and 118 cases (43.1%) were referral cases. The latitudes in QLD ranged from 19.29S to 28.04S. The latitudes in WA ranged from 31.29S to 33.59S. The latitudes in SA ranged from 34.16S to 35.85S. The latitudes in VIC ranged from 37.13S to 38.25S. The latitude in TAS ranged from

42.30S to 43.13S. Details about the climate in each location are provided in Supplementary Items 1-4.

The year of diagnosis ranged from 2002 to 2018 with significantly more cases diagnosed in the second half of the study (2 cases/year [1 – 8] vs. 19 cases/year [6 – 79], p = 0.01).

The age at diagnosis (based on 218 cases) ranged from 8 to 42 years with a median of 21 years. The median body weight (based on 123 cases) was 375 kg ranging from 120 to 639 kg. Sex (based on 255 cases) included 148 (58%) males, of which 136 (88.3%) were geldings, and 107 females (42.0%). Based on 262 cases, breeds included 33 Thoroughbreds (25.2%), 27 Warmbloods (20.6%), 25 Arabs (19.1%), 19 Quarter-Horse-related breeds (14.5%), 14 Australian Stock horses (10.7%), 9 Standardbreds (6.9%), 3 draughts (2.3%) and one other horse (0.8%) making 136 horses (51.9%) as well as 50 mixed breed ponies (39.7%), 26 Welsh ponies (20.6%), 17 Australian Riding Ponies (13.5%), 24 Shetlands (19.0%), 8 Miniature ponies (6.3%) and one Connemara (0.8%) making 126 ponies (48.1%).

Season of diagnosis was documented in 273 cases (99%). Sixty-one cases (22.3%) were diagnosed in spring, 52 cases (19.0%) in summer, 105 cases (38.5%) in autumn and 55 cases (20.1%) in winter.

A primary complaint (based on 217 cases) included 117 cases (53.9%) with lameness and 37 (17.1%) with gastrointestinal disorders. The duration of the complaint (based on 151 cases) ranged from one day to 12 years with a median of 182 days. The initial owner-reported clinical sign (based on 192 cases) included 104 cases (54.2%) of lameness, 24 cases (12.5%) of gastrointestinal signs and 20 cases (10.5%) of abnormal coat shedding. A history of chronic disorders (based on 216 cases) was present in 158 cases (73.1%) including 96 cases (60.8%) of chronic lameness, 34 cases (21.5%) of chronic gastrointestinal disorders, 21 cases (13.3%) of chronic respiratory diseases, 18 cases (11.4%) of chronic ophthalmologic disorders and 16 cases (10.1%) of dermatologic conditions. An infectious process was suspected in 59 cases (38.8%).

Among the signalment and history data, being a pony, higher body condition score, younger age and year of diagnosis were associated with survival (Table 1), latitude, lower ambient temperature during month of diagnosis and complaint of lameness were associated with a diagnosis of laminitis (Table 2) and referral, being a pony as well as history of a chronic disease were associated with a diagnosis of ID (Table 3).

Physical examination

Physical examination was partially documented in 176 cases (64.2%). The most commonly reported anomalies were hypertrichosis (129 cases, 73.3%), lameness (129 cases, 66.5%),

tachypnoea (73 cases, 51.4%), muscle loss (71 cases, 51.1%), lethargy (75 cases, 48.7%), abnormal fat distribution (61 cases, 44.5%), weight loss (79 cases, 44.9%), pendulous abdomen (45 cases, 37.5%), abnormal perspiration including hyperhidrosis or anhidrosis (24 and 14 cases respectively adding up to 36.9%) and polyuria/polydipsia (30 cases, 26.8%).

Latitude was a predictor of clinical signs of PPID with increased odds of having polyuria/polydipsia (1.08 [1.01 – 1.16], p = 0.03) and anhidrosis (1.33 [1.18 – 1.52], p = 0.01) at lower latitudes. Climatic factors also had a significant effect on the clinical presentation of PPID with hypertrichosis diagnosed during shorter days (11.47 [9.58 – 14.72] hours of daylight vs. 12.42 [9.82 – 15.27] hours of daylight, p = 0.01) and on more humid days (62 [31 – 73] % humidity vs. 52 [31 – 72] % humidity, p = 0.01), and anhidrosis diagnosed on days with higher ambient temperature (27.1 [22.0 – 31.2] ^{o}C vs. 20.2 [13.1 – 31.2] ^{o}C, p = 0.01), higher rainfall (70.15 [159 – 296.4] mm vs. 53.1 [19.9 – 269.4] mm, p = 0.03) and higher humidity (64 [55 – 73] % vs. 55.5 [31 – 68] %, p = 0.01).

Age was also a predictor of clinical signs of PPID with older animals showing increased odds of weight loss (1.14 [1.07 – 1.21], p = 0.01), lethargy (1.10 [1.04 – 1.17], p = 0.01), muscle loss (1.14 [1.07 – 1.22], p = 0.01), pendulous abdomen (1.19 [1.10 – 1.30], p = 0.01), hyperhidrosis (1.12 [1.03 – 1.23], p = 0.01) and decreased odds of presenting with lameness (0.87 [0.82 – 0.93], p = 0.01).

Veterinary diagnosis of an infection was documented in 84 of 180 cases (46.7%): 25 cases (29.8%) involving the respiratory system, 23 cases (27.4%) the gastrointestinal system, 15 cases (18.1%) the ocular system, 20 cases (24.1%) the locomotor system and 10 cases (12.0%) the integumentary system.

Among the physical examination data, absence of muscle loss, polyuria/polydipsia, hyperhidrosis and alimentary tract infection were associated with survival (Table 1) and lameness was associated with a diagnosis of laminitis (Table 2). No physical examination findings were associated with a diagnosis of ID.

Diagnostic testing

An endogenous ACTH concentration was used in 263 cases (96%), a TRH stimulation test was used in 12 cases (4.4%), an ODST was used in 9 cases (3.3%) and 2 cases (0.7%) were diagnosed at necropsy.

Insulin dysregulation was investigated in 68 cases (24.8%) and diagnosed in 52 cases (76.5%). Endogenous insulin was used in 31 cases (62.0%) and positive in 24 cases (77.4%). An OGT was used in 26 cases (52.0%) and positive in 20 cases (76.9%). A 2-step insulin-response test was used in 11 cases (22%) and positive in 8 cases (72.7%).

Laminitis was investigated in 88 cases (32.1%) and diagnosed in 79 cases (89.8%). Among those 79 laminitic cases, radiographs were used in 78 cases (98.7%) and necropsy was used in one case (1.3%). Thirty-six cases had laminitis in all four feet (45.6%), 33 in both front feet only (41.7%) and 10 in one foot only (12.7%).

Serum biochemistry and complete blood count were partially documented in 89 cases (32.5%). Nineteen cases (29.7% of 64 cases) presented with lymphopenia ($<1.10 \times 10^9$/L), 15 cases (25.0% of 60 cases) presented with neutrophilia ($>8.00 \times 10^9$/L), 15 cases (24.6% of 61 cases) presented with hypophosphatemia (<0.60 mmol/L) and 15 cases (20.3% of 74 cases) presented with hyperglycaemia (>9.0 mmol/L).

Parasite burden was recorded in 27 cases (9.9%) and revealed a faecal egg count ≥200 eggs/g in 18 cases (66.7%).

Among the diagnostic testing data, phosphorous, neutrophil count and absence of neutrophilia were associated with survival (Table 1) and endogenous ACTH was associated with a diagnosis of ID (Table 3). No diagnostic testing data were associated with a diagnosis of laminitis. Interestingly, a diagnosis of laminitis was not associated with survival ($p = 0.1$) and a diagnosis of ID was not associated with either survival ($p = 0.1$) nor a diagnosis of laminitis ($p = 0.6$).

Treatment

Pergolide was prescribed in 218 of 247 cases in which medications were documented (88.3%). The initial dose of pergolide ranged from 0.5 to 12.5 µg/kg with a median of 2 µg/kg. The type of pergolide initially used was known in 119 cases (54.6%), of which 54 cases (45.6%) were started on a liquid form and 65 cases (54.6%) on a tablet form. The final dose of pergolide ranged from 0.5 to 16 µg/kg with a median of 2 µg/kg, with 61 cases (36.5%) for which the dose was increased. The type of pergolide used at the final recheck was known in 73 cases (33.5%), of which 16 cases (21.9%) were on a liquid and 57 cases (78.1%) on a tablet form. Changes between the tablet and liquid forms was documented in 14 cases (6.4%) where 12 cases (85.7%) changed from the liquid to the tablet and 2 cases (14.3%) changed from the tablet to the liquid.

Metformin was prescribed in 12 cases which all received pergolide treatment as well. Additional recommendations (based on up to 139 cases) included 50 cases (86.2%) with dietary modifications, 96 cases (75.0%) with corrective shoeing and trimming and 24 cases (24.7%) which had the coat clipped.

Among the therapeutic data, administration of pergolide was associated with survival (Table 1). No therapeutic variable was associated with a diagnosis of laminitis or ID.

Follow-up and survival

Follow-up was available in 197 cases (71.9%). Among those cases, 137 (69.5%) were alive at last recheck and considered as survivors. Follow-up ranged from 0 (euthanasia at diagnosis) to 85 months, with a median time of 11 months. Clinical improvement, as per attending clinician, at recheck was observed in 93 out of 159 cases in which it was documented (58.5%). Ninety-two cases treated with pergolide revealed clinical improvement (64.3%) as well as one case without pergolide treatment (6.7%).

Retesting was performed in 124 cases (62.9% of 166 cases) and time to retest ranged from one to 64 months with a median of 4 months. In 43 of those 124 cases (34.7%), all on pergolide, ACTH was within reference intervals. A second retest was performed in 49 of 124 cases that had a first retest (39.5%) and time to second retest ranged from 2 to 60 months with a median of 12.5 months. In 11 of those 49 cases (22.4%), all on pergolide, ACTH was within reference intervals. A third retest was performed in 24 of 49 cases that had a second retest (49.0%) and time to retest ranged from 4 to 67 months with a median of 20 months. In 6 of those 24 cases (25%), all on pergolide, ACTH was within reference intervals. A fourth retest was performed in 11 of 24 cases that had a third retest (45.8%) and time to retest ranged from 18 to 59 months with a median of 25 months. In 2 of those 11 cases (18.2%), both on pergolide, ACTH was within reference intervals.

Overall, 52 horses of the 124 that had retesting (41.9%) had normal endogenous ACTH at rechecks and all of those received pergolide. Among 117 horses that received pergolide and were retested, 52 (44.4%) had normal endogenous ACTH at rechecks, including 14 (26.9%) of which that required a dose increase. Among horses not receiving pergolide, none had normal endogenous ACTH at rechecks.

Among the follow-up data, presence of clinical improvement at recheck noted by a veterinarian was associated with survival (Table 1). No follow-up variable was associated with a diagnosis of laminitis or ID.

Multivariable analysis

Due to missing data, logistic regression was confined to the variables associated with survival. Backward stepwise logistic regression indicated that higher body condition score, being a pony and administration of pergolide were independently associated with survival (Table 4).

Discussion

The main findings of this study indicate that being a pony, maintaining a higher body condition score and administering pergolide were associated with survival. The clinical presentation of PPID changes with latitude and climate and ID is commonly diagnosed in equids with PPID.

Although PPID is manageable, it carries a relatively poor prognosis [10]. In our study, prognosis was worse than previously described in other referral practices as only 69.5% survived with a median follow-up of 11 months [10]. A possible reason for this higher value is the late presentation of the cases. Only 50% were presented in the absence of lameness, suggesting that other clinical signs had not been identified, or had been considered to be "normal" age-related changes by the owners. Close to 90% of horses that had radiographs had changes consistent with laminitis. This frequency is also higher than previously reported and is consistent with the fact that, in our study, veterinary care was only sought when horses were lame [10; 27].

Being a pony was associated with survival. There is conflicting evidence for differences in hormone levels between horses and ponies, and while some studies identified higher ACTH and α-MSH in ponies, current reference intervals do not discriminate on the basis of breed [4; 13; 28; 29]. Most ponies in the present study showed clinical signs consistent with PPID and there was no difference in ACTH concentrations between horses and ponies [29]. Interestingly, ponies were also more likely to suffer from ID. This finding is consistent with other studies; however, as ID has been clearly associated with laminitis, including in cases of PPID, one could expect a negative association with survival [30-32]. This was not the case, as no association could be found between ID and survival, laminitis and survival and ID and laminitis. The reason for this lack of association could be the low number of horses in which ID was investigated and the strict inclusion criteria for laminitis limiting the number of horses in which both could be documented, thereby weakening the statistical analysis.

As previously reported, loss of body condition was a common sign of PPID and in our study, a higher body condition score was associated with survival [10]. The increased likelihood of PPID horses to suffer from endoparasitism could also exacerbate the observed weight loss as many horses in our study had a heavy parasite burden [33]. Unlike other studies, neutrophilia was a common feature of cases with PPID [2]. Although neutrophilia has been found not to be associated with PPID, it has been associated with the presence of chronic diseases in PPID cases [2; 10]. As neutrophils from PPID cases have been shown to have a decreased activity, the higher neutrophil count in non-survivors could be explained by a higher rate of chronic

infections in more severe PPID cases leading to poor body condition score and poor outcome [34].

A lower latitude was associated with increased odds of developing polyuria/polydipsia and anhidrosis. Distance from the equator affects daylength, temperature, rainfall and humidity (Supplementary Items 1-4), but there are also conflicting data regarding its effect on the POMC-derived peptide circannual amplitude with some studies suggesting that more severe POMC-derived peptide-induced effects could be seen at higher latitudes and other suggesting the opposite [13; 14]. Nevertheless, the link between POMC-derived peptide concentration and clinical signs is still unknown. Polyuria/polydipsia in PPID cases could be explained by reduced antidiuretic hormone secretion from the pars nervosa due to the compression of the pars intermedia and is usually seen in more severe cases [1]. Anhidrosis has been previously reported in locations closer to the equator with warmer and more humid climates, and our results are consistent with that [15]. Hyperhidrosis is a recognised clinical sign associated with PPID, but is likely worsened in hot climates with an increased risk of sweat gland exhaustion and resultant anhidrosis. This association between anhidrosis and latitude is likely to occur in any warm climate rather than a consequence of being in Australia; therefore, in warm humid climates of south-eastern USA, anhidrosis is anecdotally a sign of PPID. That being said, the climatic data should be interpreted carefully as PPID is a chronic progressive disease and climatic values at time of diagnosis may not reflect the overall climate under which the horse developed its clinical signs.

Insulin dysregulation, a key component of EMS, has previously been reported in about 30% of cases diagnosed with PPID [2]. In our study, ID was only investigated in less than 25% of horses; however up to 76.5% of those were diagnosed as insulin dysregulated. The apparent discrepancy could be caused by the strict inclusion criteria for a diagnosis of ID and by a bias in our retrospective study where, on one hand, horses suspected of EMS based on morphometric appearance might not have received ID testing and, on the other hand, only cases suspected of EMS might have been tested. In favour of the latter, ID was more commonly diagnosed in referred cases, which would be consistent with more complex cases not responding to treatment due to multiple endocrinopathies. Interestingly, endogenous ACTH was higher in horses with ID. Although EMS and PPID are different entities, there have been some reports of cross-talk between PPID and EMS as increases in α-MSH and CLIP can increase insulin secretion suggesting that severe cases of PPID with high hormone concentrations would be more likely to have ID [35; 36]. Although our assessment of ID was

limited by the low number of cases in which ID was investigated, our finding strongly suggests that, in all cases of PPID, ID should be investigated, especially as it is suspected that PPID-associated laminitis might be a consequence of hyperinsulinaemia [31].

Treatment with pergolide was strongly associated with survival. This finding is consistent with previous reports that established pergolide as the treatment of choice for PPID [10; 16; 37]. In Australia, two forms of pergolide are approved for use in horses, a liquid form and a tablet form. Both products were initially prescribed at the same frequency and no statistical difference in ACTH values or pergolide doses were found at first retest; however, at final retest, up to 78% of the horses were treated with pergolide as a tablet. Although no conclusion could be drawn, this finding could suggest that, as previously described, the tablet form of pergolide would be more suitable for long-term treatment [38].

The main limitation of our study is the lack of uniform testing at all locations resulting in low number of PPID cases in which a diagnosis of laminitis and ID was attempted and preventing a robust statistical analysis of those outcomes. In addition, the definition and the awareness of PPID amongst owners and veterinarians has changed over the course of the study with changes in diagnostic tests and improved reference ranges being developed. Nevertheless, as previously described, PPID is an increasingly diagnosed disease in older horses [10; 17]. Our study emphasises that close attention to subtle clinical signs and treatment with pergolide is paramount for improved survival and that ID should be investigated in PPID cases. This study further supports that preventive equine veterinary care should include monitoring of metabolic and endocrine health as well as client education.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Research ethics committee oversight not required by this journal: descriptive clinical report. Explicit owner informed consent for inclusion of animals in this study was not stated.

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None.

Authorship

R. Horn and F.R. Bertin designed the study; all co-authors collected data; F.R. Bertin analysed the data; R. Horn and F.R. Bertin interpreted the findings; R. Horn and F.R. Bertin prepared the manuscript; all co-authors contributed and approved the final version of the manuscript.

Manufacturers' addresses

[a]Prism, GraphPad Software, Inc. La Jolla, California, USA.

[b]IBM SPSS Statistics 24, IBM Corp. Armonk, New York, USA.

Tables

Table 1: Univariable analysis for variables associated with survival in horses diagnosed with PPID. Only variables that were significantly different ($p<0.05$) between groups are presented.

Variable	Survivors (value [range] or percentage)	Non-survivors (value [range] or percentage)	p-value
Pony (n = 194)	54.1%	35.6%	0.02
Age (years, n = 185)	20 [9 – 38]	23 [8 – 39]	0.01
Year of diagnosis (n = 196)	2016 [2002 – 2018]	2014 [2002 – 2018]	0.01
BCS (/5, n = 115)	3 [1.5 – 5]	2 [0.5 – 4.5]	0.01
Muscle loss (n = 122)	44.1%	71.1%	0.01
PUPD (n = 98)	20.3%	41.4%	0.03
Hyperhidrosis (n = 94)	13.4%	44.4%	0.01
Alimentary system infection (n=66)	15.0%	42.3%	0.01
Phosphorus (mmol/L, n = 48)	0.9 [0.5 – 2.1]	0.8 [0.5 – 1.2]	0.01
Neutrophil count ($x10^9$/L, n = 47)	4.9 ± 1.8	7.8 ± 3.0	0.01
Neutrophilia (n = 47)	9.1%	50.0%	0.01
Pergolide (n = 191)	94.8%	80.7%	0.01
Clinical improvement at recheck (n = 159)	82.7%	10.9%	0.01

Table 2: Univariable analysis for variables associated with a diagnosis of laminitis in horses diagnosed with PPID. Only variables that were significantly different ($p<0.05$) between groups are presented.

Variable	Laminitis (value or percentage)	No laminitis (value or percentage)	p-value
Latitude (Abs°, n = 88)	35.07 [19.29 – 38.26]	27.39 [19.29 – 34.64]	0.01
Complaint of lameness (n=87)	89.7%	44.4%	0.01
Average ambient temperature (°C) during month of diagnosis (n = 88)	23.3 [13.1 – 31.2]	27.1 [15.8 – 31.2]	0.03
Lameness (n = 81)	95.6%	57.1%	0.01

Table 3: Univariable analysis for variables associated with a diagnosis of ID in horses diagnosed with PPID. Only variables that were significantly different ($p<0.05$) between groups are presented.

Variable	Insulin dysregulation (value or percentage)	No insulin dysregulation (value or percentage)	p-value
Referral (n = 68)	51.9%	18.6%	0.02
Pony (n = 68)	67.31%	18.8%	0.01
History of chronic disorder (n = 59)	75.6%	42.9%	0.04
Basal ACTH (pg/mL, n = 66)	120 [18.5 – 1250]	72.2 [32.9 – 770]	0.02

Table 4: Results of backward stepwise logistic regression analysis of variables as predictors of survival in horses with PPID. Horses were categorised as survivors (51 horses) or non-survivors (20 horses). The Hosmer–Lemeshow Goodness-of-Fit test indicated a good fit ($p = 0.23$) to the logistic regression model. *Body condition score was analysed as an ordinal categorical variable and then considered as a covariate.

Variable	Estimate	S.E.	p-value	Odds ratio	95% CI

Intercept	-1.73	0.87	0.04		
BCS (/5)*	0.94	0.30	0.01	2.56	1.44 – 4.55
Pony	1.07	0.53	0.04	2.92	1.03 – 8.22
Pergolide	-1.34	0.66	0.04	3.83	1.06 – 13.89

Figure legend

Fig 1: Repartition of cases across Australia. The size of the circle matches the number of cases included in the analysis.

Supporting Information

Supplementary Item 1: Monthly average day length.

Supplementary Item 2: Monthly average temperatures.

Supplementary Item 3: Monthly average rainfalls.

Supplementary Item 4: Monthly average humidity.

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TABLES

Table 1: Univariable analysis for variables associated with survival in horses diagnosed with PPID. Only variables that were significantly different ($p < 0.05$) between groups are presented.

Variable	Survivors (value [range] or percentage)	Non-survivors (value [range] or percentage)	p-value
Pony (n = 194)	54.1%	35.6%	0.02
Age (years, n = 185)	20 [9 – 38]	23 [8 – 39]	0.01
Year of diagnosis (n =196)	2016 [2002 – 2018]	2014 [2002 – 2018]	0.01
BCS (/5, n=115)	3 [1.5 – 5]	2 [0.5 – 4.5]	0.01
Muscle loss (n=122)	44.1%	71.1%	0.01
PUPD (n=98)	20.3%	41.4%	0.03
Hyperhidrosis (n=94)	13.4%	44.4%	0.01
Alimentary system infection (n=66)	15.0%	42.3%	0.01
Phosphorus (mmol/L, n=48)	0.9 [0.5 – 2.1]	0.8 [0.5 – 1.2]	0.01
Neutrophil count ($x10^9$/L, n=47)	4.9 ± 1.8	7.8 ± 3.0	0.01
Neutrophilia (n=47)	9.1%	50.0%	0.01
Pergolide (n=191)	94.8%	80.7%	0.01
Clinical improvement at recheck (n=159)	82.7%	10.9%	0.01

Table 2: Univariable analysis for variables associated with a diagnosis of laminitis in horses diagnosed with PPID. Only variables that were significantly different ($p < 0.05$) between groups are presented.

Variable	Laminitis (value or percentage)	No laminitis (value or percentage)	p-value
Latitude (Abs^o, n=88)	35.07 [19.29 – 38.26]	27.39 [19.29 – 34.64]	0.01
Complaint of lameness (n=87)	89.7%	44.4%	0.01
Average ambient temperature (^oC) during month of diagnosis	23.3 [13.1 – 31.2]	27.1 [15.8 – 31.2]	0.03

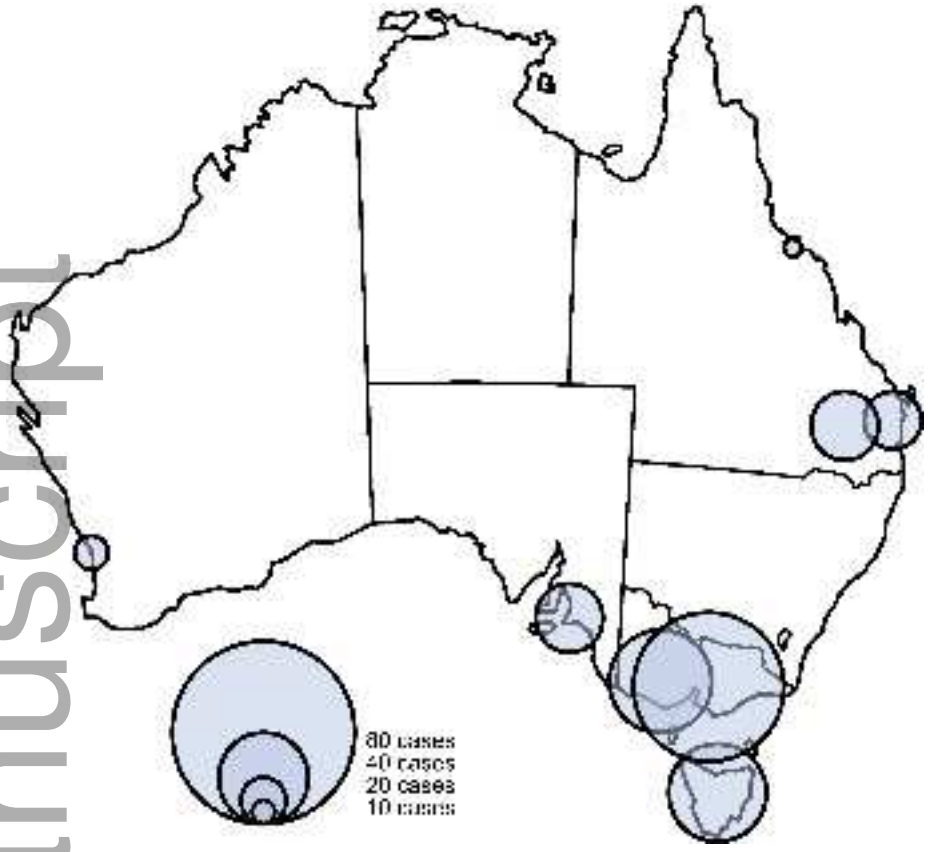
(n = 88)			
Lameness (n=81)	95.6%	57.1%	0.01

Table 3: Univariable analysis for variables associated with a diagnosis of ID in horses diagnosed with PPID. Only variables that were significantly different ($p < 0.05$) between groups are presented.

Variable	Insulin dysregulation (value or percentage)	No insulin dysregulation (value or percentage)	p-value
Referral (n=68)	51.9%	18.6%	0.02
Pony (n=68)	67.31%	18.8%	0.01
History of chronic disorder (n=59)	75.6%	42.9%	0.04
Basal ACTH (pg/mL, n=66)	120 [18.5 – 1250]	72.2 [32.9 – 770]	0.02

Table 4: Results of backward stepwise logistic regression analysis of variables as predictors of survival in horses with PPID. Horses were categorized as survivors (51 horses) or non-survivors (20 horses). The Hosmer–Lemeshow Goodness-of-Fit test indicated a good fit ($p = 0.23$) to the logistic regression model. *Body condition score was analysed as an ordinal categorical variable and then considered as a covariate.

Variable	Estimate	S.E.	p-value	Odds ratio	95% CI
Intercept	-1.73	0.87	0.04		
BCS (/5)*	0.94	0.30	0.01	2.56	1.44 – 4.55
Pony	1.07	0.53	0.04	2.92	1.03 – 8.22
Pergolide	-1.34	0.66	0.04	3.83	1.06 – 13.89



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