Received: 16 August 2018 | Accepted: 9 January 2019
DOI: 10.1111/jvim.15419

Journal of Veterinary Internal Medicine Open Access
ACVIM American College of Veterinary Internal Medicine

STANDARD ARTICLE

Phenotypic, hormonal, and clinical characteristics of equine endocrinopathic laminitis

Melody A. de Laat[1] | Martin N. Sillence[1] | Dania B. Reiche[2]

[1]School of Earth, Environmental and Biological Sciences, Queensland University of Technology (QUT), Brisbane, Queensland, Australia

[2]Animal Health, Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany

Correspondence
Melody A. de Laat, School of Earth, Environmental and Biological Sciences, Queensland University of Technology (QUT), Brisbane, Queensland 4000, Australia.
Email: melody.delaat@qut.edu.au

Funding information
Boehringer Ingelheim

Background: Equine endocrinopathic laminitis is common and can be associated with an underlying endocrinopathy, such as equine metabolic syndrome (EMS), pituitary pars intermedia dysfunction (PPID), pasture consumption, or any combination of these factors.

Objectives: The aim of the study was to improve the risk assessment capabilities of clinicians, and to inform management strategies, for acute endocrinopathic laminitis by prospectively examining the phenotypic, hormonal, and clinical characteristics of the disease in a large cohort.

Animals: Privately owned horses and ponies (n = 301) of any age, sex, or breed diagnosed with laminitis by a veterinarian. A history of laminitis was acceptable.

Methods: This was a prospective cohort study. Veterinarians provided information on each case via an online questionnaire after informed consent from the animal's owner, and all data were de-identified before analysis. Serum insulin and plasma adrenocorticotrophic hormone concentrations were measured in each case.

Results: Most cases were recruited in spring (109/301; 36.2%). Concurrent EMS and PPID resulted in higher basal insulin concentrations (49 [21.5-141]; $P < .02$) than if an animal had a single underlying cause for their laminitis. The insulin concentration was negatively correlated ($r^2 = -0.38$; $P < .001$) with the animal's height, being higher in ponies (33[10-14]; $P < .001$) than horses (9.5 [3-25.7]) and was positively correlated ($r^2 = 0.12$; $P = .05$) with their grade (severity) of laminitis.

Conclusions and Clinical Importance: Horses and ponies with concurrent endocrinopathies have more marked hyperinsulinemia. Higher basal insulin concentrations were associated with more severe lameness.

KEYWORDS

ACTH, equine metabolic syndrome, horse, hyperinsulinemia, pituitary pars intermedia dysfunction

1 | INTRODUCTION

Equine laminitis is an important disease affecting the attachment of the distal phalanx to the inner hoof wall,[1] and the endocrinopathic form of the disease occurs most commonly.[2,3] Although previous research has focused on the inflammatory forms of the disease, recent data support that endocrinopathic laminitis is a worldwide welfare issue that is presented to equine practitioners reasonably frequently.[4–6] Currently, data on the clinical features of laminitis specific to the endocrinopathic form of the disease are limited, and owners might fail to recognize the disease, particularly in early or mild cases.[7,8]

Endocrinopathic laminitis is associated with hyperinsulinemia[6,9] and is a frequent sequelae to 2 common equine endocrinopathies: equine metabolic syndrome (EMS) and pituitary pars intermedia dysfunction (PPID).[10,11] Increased adiposity (generalized or regional, or both) is also a risk factor for the disease.[12] Pasture-associated cases of laminitis are associated with hyperinsulinemia (without any accompanying signs of inflammatory processes, such as diarrhea) and therefore are also likely to often fall under the umbrella of endocrinopathic laminitis.[13,14]

Abbreviations: ACTH, adrenocorticotropic hormone; BCS, body condition score; CNS, cresty neck score; EMS, equine metabolic syndrome; NSC, nonstructural carbohydrate; OGT, oral glucose test; OST, oral sugar test; PPID, pituitary pars intermedia dysfunction.

Hyperinsulinemia can be transient and occur in response to carbohydrate consumption, or it can be persistent, as indicated by basal (resting) hyperinsulinemia, which might result from, or induce, tissue resistance to insulin.[15,16] Resting hyperinsulinemia, insulin resistance, and abnormal insulin responses to oral carbohydrate can all occur in an insulin-dysregulated horse or pony (alone or in combination), and any uncontrolled hyperinsulinemia increases an individual's risk of laminitis.[9,17]

Given the number of potential underlying causative factors, or combinations thereof, it is important to gain an increased understanding of the complex interplay among these contributing factors in endocrinopathic laminitis. These data would then aid in the understanding, diagnosis, and management of what is currently a challenging condition for practitioners to treat and prevent.

The current study sought to prospectively examine a cohort of horses and ponies with endocrinopathic laminitis to improve our knowledge of the factors contributing to disease. The specific aim of the study was to describe the phenotypic, hormonal, and clinical characteristics of acute endocrinopathic laminitis in animals diagnosed with the condition by a veterinarian. A secondary aim of the study was to examine whether sampling horses/ponies for insulin concentration at the time of laminitis diagnosis (before treatment) results in a higher insulin concentration than animals sampled after treatment has commenced.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a prospective cohort study of acute endocrinopathic laminitis that accepted self-enrolled cases over a 2-year period from the 1st of January 2014 to the 31st of December 2015. All participants were privately owned horses and ponies that had been diagnosed with acute laminitis by a veterinarian within 4 weeks of entry into the study. A history of laminitis did not preclude an animal from inclusion in the study. The recruits could be of any age, sex, or breed, although donkeys were excluded. Informed consent for involvement in the study was provided by the owner of the animal, and all data were de-identified on entry to the study before analysis. The study was approved by the Human and Animal Ethics Committees of Queensland University of Technology (1600000936, 1300000744).

The attending veterinarian who diagnosed the case of laminitis was required to complete an online questionnaire about the horse/pony, and the nature of the laminitis (Supplementary Item 1). The questions encompassed the history, signalment, clinical examination findings, Obel grade of lameness,[18] animal management, and veterinary treatments for each case. The questionnaire was accessed and submitted electronically via a designated study website. Extensive information on body condition scoring as well as interpretive guidelines for digital radiography were available to the veterinarians on the study website to encourage consistency of reporting.

For each animal basal (resting) plasma, adrenocorticotropic hormone (ACTH) and serum insulin concentrations were measured upon entry to the study. Sample submission packs were sent to recruiting veterinarians, and the samples were couriered to a commercial laboratory on ice within 4 hours of collection. It was requested that samples were collected from a fasted animal, although the recent ingestion of pasture or a hay-type forage was acceptable. Furthermore, veterinarians submitting samples were encouraged to ensure that the horse/pony was not in pain at the time of blood sampling in an effort to reduce any effect of pain on serum insulin concentration. In many cases, the submitting veterinarian/farrier was able to take the blood sample from the horse/pony at a follow-up visit to address this request, although this was not possible in all cases. As such, the timing of the blood sample was taken into consideration when examining the serum insulin concentration.

The samples were analyzed at 1 of 3 commercial laboratories: VetPath Laboratories, Epsom, WA, Australia; Animal Health Laboratories, Cornell, New York; and Liphook Equine Laboratory, Hampshire, United Kingdom. The exclusive use of these laboratories aimed to ensure that sample handling was consistent and that minimal variation with respect to laboratory assays occurred. The same chemiluminescent assay (Immulite; Siemens Healthcare, Tarrytown, NY, USA) was used for the analysis of ACTH at all laboratories, as was the case for insulin at VetPath and Liphook, who analyzed the majority of the insulin samples. A small number of insulin samples (4%) was analyzed using a radioimmunoassay (Siemens Healthcare) at Cornell. These human-specific assays have been adapted for use in the equine species and validation data have been published previously.[19,20] However, because of differences between the 2 insulin assays, the insulin data from samples analyzed at Cornell have been removed from the statistical analyses (but were used for case definition).

2.2 | Case definition

The submission data for each case of laminitis were examined to ensure that the laminitis was likely to be endocrinopathic in origin. A set of inclusion criteria was developed and if the case exhibited 2 or more of the inclusion criteria, or 1 of the criteria in the absence of an obvious nonendocrine initiating cause for laminitis, they were deemed acceptable for inclusion in the final data set. The inclusion criteria included an increased basal (resting) serum insulin concentration (>20 μIU/mL), an increased plasma ACTH concentration (>35 pg/mL or seasonally adjusted, location-specific reference ranges if the sample was obtained during the autumn period, which were 47 pg/mL for the Northern hemisphere,[19] and 77.4 pg/mL for the Southern hemisphere[21]), a body condition score (BCS) ≥ 6/9, a cresty neck score (CNS) ≥ 2/5, regional adiposity, a history of recent access to lush pasture, a change in diet that included an increase in nonstructural carbohydrate (NSC) content without accompanying clinical signs of gastrointestinal disturbance (ie, not a sudden overload of grain with diarrhea, colic, acidosis, fever, lethargy, etc.), being a breed at-risk of developing EMS,[10] a history of being an easy-keeper, founder (divergent growth) rings in 1 or more hoof wall, a familial history of laminitis, a dysregulated postprandial serum insulin response to an oral glucose test (OGT) or an oral sugar test (OST), with the choice of dynamic test dependent on the clinician's preference. When this test was performed, a diagnostic cutoff of >60 μIU/mL 60 minutes after the meal was used for the OST,[22] whereas a cutoff value of >80 μIU/mL 2 hours after the meal was used for the OGT.[23]

Each case of endocrinopathic laminitis was classified as being associated with EMS, pasture consumption, PPID, or any combination of these 3. The criteria used to identify the cases of EMS included increased resting (>20 μIU/mL) or postprandial (>60 μIU/mL for OST and >80 μIU/mL for OGT) serum insulin concentration, a cresty neck (score ≥ 2/5), and evidence of regional adiposity or increased BCS (≥6/9), or both. A history of new access to pasture was used to identify pasture-associated cases, and PPID cases were identified based on an increased plasma ACTH concentration (>35 pg/mL or seasonally adjusted as per the inclusion criteria). Eleven animals were being treated with pergolide mesylate at the time they were recruited to the study, and a diagnosis of PPID was recorded based on historical data in these cases. The ACTH data from these animals were not included in the ACTH analyses.

2.3 | Data analyses

The data were normality tested using a Shapiro-Wilk test and where normally distributed were analyzed parametrically. All nonparametric data were analyzed with nonparametric tests. Thus, the data are presented as either mean ± SE or median (interquartile range). Missing values were ignored, thus the *n* value for any given statistical outcome might not equal the total sample size. Simple comparisons of groups within the whole cohort, for example, male versus female, were undertaken with an unpaired *t*-test or the Mann-Whitney rank sum test. Multiple variables were analyzed with a Kruskal-Wallis analysis of variance (ANOVA) on ranks with Dunn's post hoc test. Spearman's correlation coefficient was used to examine the linear relationship between 2 variables. The relationship between 2 categorical variables (eg, the Obel grade of laminitis and the number of feet affected) was assessed with a Chi-square test. Significance was accepted at $P \leq .05$. The data were analyzed with SigmaPlot v.13 (Systat Software, San Jose, California).

3 | RESULTS

3.1 | Case recruitment

A total of 301 cases of laminitis met the inclusion criteria and were included in the study. Cases from both the Southern (Australia and New Zealand) and Northern (United States, United Kingdom, and Germany) hemispheres were included, although the majority of cases were recruited in Australia. The cohort of 151 males and 150 females included 115 horses (>144 cm) and 186 ponies (≤144 cm), with a mean age of 15.6 ± 0.4 years. Nineteen different breeds of horse/pony were recorded and included Shetland pony (17.3%), Australian pony (13.3%), Quarter horse (12.6%), Welsh pony (12%), Warmblood (7.3%), Arabian (6.3%), Thoroughbred (5.6%), Appaloosa/Palouse (3.7%), Australian stock horse (3.7%), Draught (1%), New Forest (0.7%), Standardbred (0.7%), Highland pony (0.7%), American Saddlebred (0.3%), Connemara (0.3%), Fjord (0.3%), Morgan (0.3%), Palomino (0.3%), and Tennessee Walking horse (0.3%). A number (13.3%) of participants had their breed listed as "other."

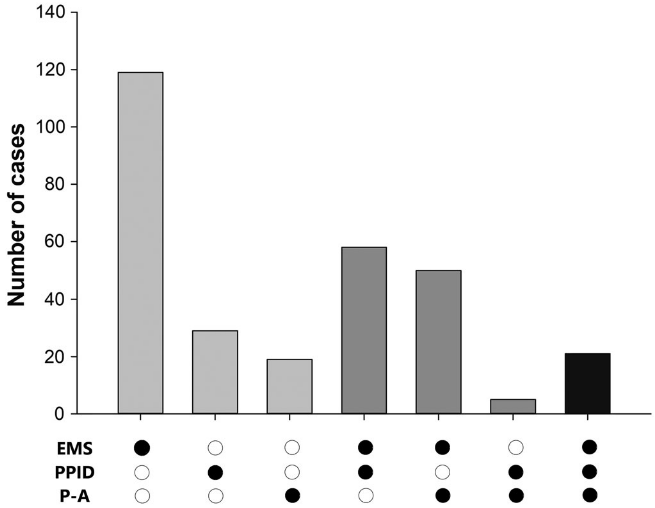

FIGURE 1 Cases of acute laminitis were diagnosed with either equine metabolic syndrome (EMS), pituitary pars intermedia dysfunction (PPID), a pasture-associated (P-A) cause (light gray bars), a combination of 2 of these factors (dark gray bars), or all 3 (black bar). Many of the cases were diagnosed with EMS, whereas PPID and P-A were less common

The most frequent endocrinopathy in participants was EMS (n = 119, 40%), with a further 58 cases (19%) presenting with evidence of both EMS and PPID (Figure 1). Another 50 cases were determined to have EMS in addition to evidence of a pasture-associated cause of the laminitis and 21 cases presented with a combination of all 3 causalities (EMS, PPID, and pasture-associated), which meant that EMS played a role in 82.4% of the cases of endocrinopathic laminitis. By comparison, PPID was a less frequent causative factor (n = 29), with an additional 5 cases of PPID occurring concurrently with a pasture-associated factor (Figure 1). Pasture-associated laminitis occurring without evidence of an underlying endocrinopathy was less common (n = 19, 6% of cases).

3.2 | Phenotypic characteristics

Consistent with their use as selection criteria, the median BCS (6 [5-7]) and CNS (3 [2, 3]) were above the ideal scores for healthy animals.[24,25] In addition, BCS and CNS were positively correlated ($r^2 = 0.48$, $P < .001$) for the cohort. However, neither BCS ($P = .55$) nor CNS ($P = .19$) were correlated with the basal insulin concentration. While excessive fat deposition around the nuchal ligament was noted in 70% of cases, evidence of regional adiposity was also recorded at additional locations in 88% of these animals and included fat depositions at some or all of the following: tail head, supraorbital fat pad, prepuce/mammary areas, and the ventral midline.

3.3 | Hormone analyses

3.3.1 | Insulin

The median basal (resting) insulin concentration of the 53 cases with concurrent PPID and EMS was 49 [21.5-141] μIU/mL, being markedly higher than that in the 24 cases with PPID alone (4.5 [2-12.8] μIU/mL; $P < .001$), the 117 cases with EMS alone (25 [5-85] μIU/mL; $P < .02$), or the 17 cases with pasture-associated laminitis alone

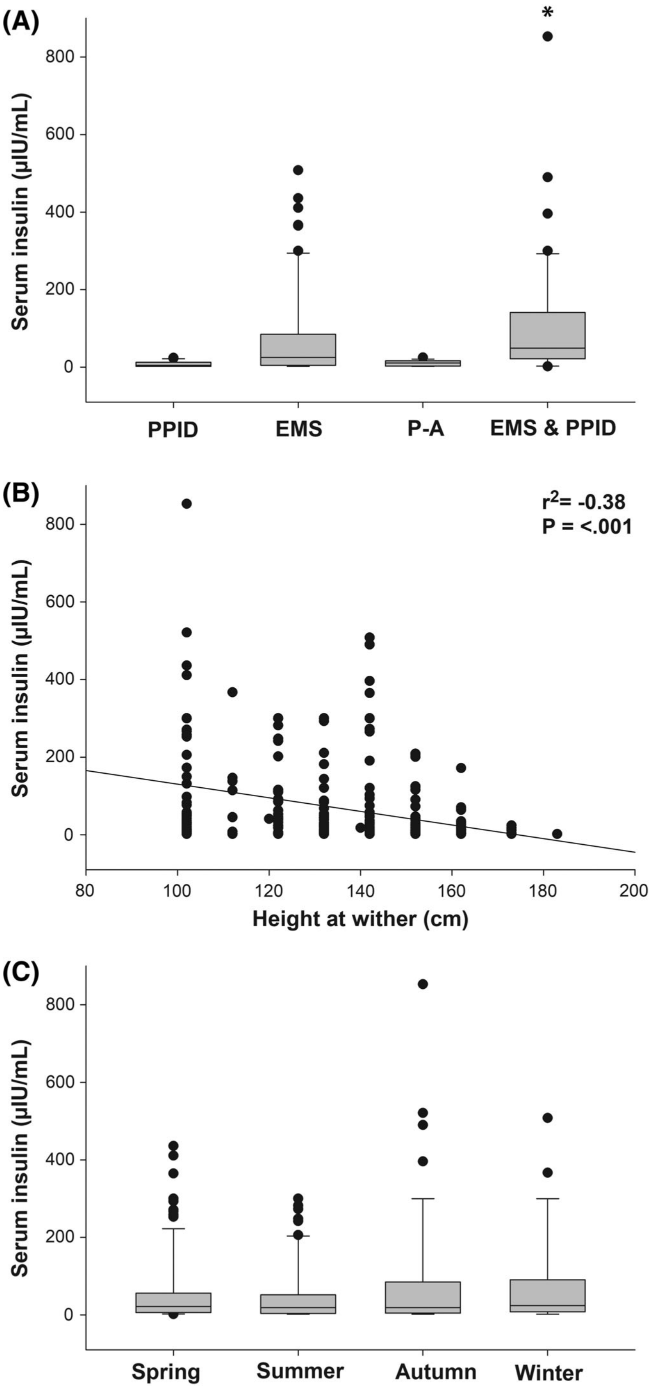

FIGURE 2 The basal (resting) serum insulin concentrations in a cohort of horses and ponies with endocrinopathic laminitis. The median insulin concentration was higher ($P < .02$) in animals with both equine metabolic syndrome (EMS) and pituitary pars intermedia dysfunction (PPID), compared to animals with EMS, PPID, or a pasture-associated cause of the disease (A). The insulin concentration was negatively correlated with height at the withers in the cohort (B); however, the insulin concentration did not differ among the seasons (C)

(11 [3-16.5] µIU/mL; $P < .01$) (Figure 2A). Although there was no effect of breed on serum insulin concentration ($F = 0.72$; $P = .8$), there was a significant ($P < .001$) negative correlation ($r^2 = -0.38$) between serum insulin concentration and height at the withers, indicating that as height increased, the resting insulin concentration decreased (Figure 2B). When further investigated as a binary variable (ie, horse versus pony), ponies were found to have higher ($P < .001$) resting serum insulin than horses (Table 1). There was no effect of season on resting serum insulin concentration (Figure 2C).

The resting serum insulin concentration did not differ between sexes (Table 1), nor with age (Table 1; $r^2 = 0.09$, $P = .12$). Insulin concentration also did not differ between animals with a history of laminitis (before entry in the study), compared to those with no history of laminitis (Table 1). Lastly, the insulin concentration in samples collected on the same day as diagnosis (31.5 [8-110] µIU/mL) was not significantly different ($P = .1$) to the insulin concentration of samples taken after treatment had been instigated (18.4 [4-45.6] µIU/mL).

3.3.2 | Adrenocorticotrophic hormone

The main variable to have a significant effect on plasma ACTH concentration was season, with a higher ($P < .001$) ACTH concentration recorded in autumn (fall) than in other seasons (Figure 3A). This was consistent for the entire cohort, and for the subset of animals diagnosed with PPID. However, plasma ACTH concentration (pg/mL) was only significantly different ($P = .01$) between autumn (26 [18-34]) and spring (15 [10-21]) in horses without PPID (Figure 3B). In autumn, the ACTH concentration was found to be higher ($P = .005$) in ponies (93 [25-367] pg/mL) than horses 30.9 [22.8-37.6] pg/mL). However, ACTH concentration did not differ according to size (horse versus pony) for the cohort as a whole (Table 1).

Given that ACTH concentration was used as a diagnostic tool for the identification of PPID, it is unsurprising that plasma ACTH concentration was higher ($P < .001$) in PPID cases (80 [47-200] pg/mL), than non-PPID cases (17 [11.5-23.2] pg/mL), and that it was higher ($P < .001$) in animals aged 16 years and above (Figure 3C). Furthermore, plasma ACTH concentration showed a positive linear correlation ($r^2 = 0.4$, $P < .001$) with age. Overall, the plasma ACTH concentration did not differ among breeds ($F = 0.61$; $P = .91$), nor was it affected by sex (Table 1). Similarly, the median plasma ACTH concentration did not differ among animals with a history of laminitis, and those that did not have a history of laminitis (Table 1).

3.4 | Descriptive epidemiology: laminitis

The greatest number of cases were recruited to the study in spring (36.2%) and summer (25.5%), with fewer cases in autumn (20.8%) and winter (17.5%). The clinical findings associated with laminitis as described by the submitting veterinarian were consistent with those classically associated with acute onset of this disease.[26] Increased digital pulses were documented in 82.4% of cases, whereas a positive response to hoof testers was apparent in 68.4%. Clinical signs associated with more chronic disease were also recorded in many cases and included divergent growth rings (60.1%), elongated or misshapen toes (39.5%), and sunken coronary bands (12%). A previous episode of laminitis (occurring before the study) was reported to have occurred in 54.4% of the participants.

The most prevalent scenarios were for laminitis to be present bilaterally in the front feet (50.3% of cases), or in all 4 ft (45.3% of cases). A period of ≥10 days had elapsed before the veterinarian being called to examine the animal in 52.8% of cases. The number of days that elapsed before the veterinarian being called were Obel grade 1;

TABLE 1 The median (IQR) basal serum insulin and plasma ACTH concentrations of horses and ponies with acute laminitis

	Insulin (μIU/mL) (n = 284)	*P*-value	ACTH (pg/mL) (n = 283)	*P*-value
Sex		.35		.68
Male	18.5 (4.0-58.0)		26 (16-51.5)	
Female	21 (6.02-70.9)		23 (13-63.3)	
Age		.30		<.001
≤15 years	18 (4.8-52.4)		18.1 (12.2-31.9)	
≥16 years	21.5 (6.8-75.5)		46 (21-118)	
Size		<.001		.25
Horse (>144 cm)	9.5 (3-25.7)		24 (14-50.9)	
Pony (≤144 cm)	33 (10-114)		25 (15-71.6)	
Previous laminitis		.22		.63
Yes	23 (7-70.9)		25 (14.4-73.6)	
No	15 (4-70.3)		26 (16-46.8)	

Abbreviations: ACTH, adrenocorticotropic hormone; IQR, interquartile range.

8.5 [3-11] days, Obel grade 2; 6 [2-11] days, Obel grade 3; 5 [2-11] days, and Obel grade 4; 2 [0-8] days. There was no correlation (r^2 = −0.13; *P* = .07) between the Obel grade of lameness and the number of days before veterinary attention was sought.

Radiographs were taken in 139 (43.8%) cases. The most common radiographic finding was evidence of rotation of the distal phalanx within the hoof capsule (77% of radiographed cases). Less common radiographic findings included decreased sole depth (45.3%), distal displacement of the distal phalanx (20.1%), increased coronary extensor distance (16.5%), evidence of gas within the soft tissues (18%), and osseous changes to the distal phalanx (40.3%).

There was a weak positive correlation (r^2 = 0.12, *P* = .05) between the serum insulin concentration and the Obel grade of lameness.[18] Given that there were only 3 cases of Obel grade 4 lameness, the Obel grade 1-3 data were also analyzed using a 1-way ANOVA on ranks. These analyses did not reveal a statistically significant (*P* = .07) difference between the median serum insulin concentration (μIU/mL) at each Obel grade of lameness: 1:15.4 [6-44.2], 2:19 [3.3-87.5], and 3:34 [8.6-112]. Lastly, there was a significant (X^2 = 20.9; *P* = .01) positive relationship between the Obel grade of lameness and the number of feet affected.

3.5 | Animal husbandry

A large proportion of the recruits (76.3%) were allowed access to pasture for grazing for longer than 3 hours per day. Unsurprisingly then, 89.3% of recruits had a pasture component to their diet. This could be broken down as 12.9% being allowed pasture only, 43.2% having a diet composed of pasture and some other form of forage, whereas 33.1% of cases were given a diet of forage, pasture access, and some concentrate. Only 8.8% were fed a diet that consisted of concentrates and non-pasture-based forage, whereas 1.9% listed their diet as "other."

4 | DISCUSSION

This study has provided a snapshot of the current epidemiological features of endocrinopathic laminitis in horses and ponies. Consistent with current understanding, the horses and ponies with acute laminitis in this study usually had a detectable endocrine disease,[27] and as expected based on the recruitment and inclusion criteria for the study, an endocrinopathy was identified in 94% of the cases. Of these endocrinopathies, EMS was the most prevalent underlying disease occurring in 82% of cases, whereas PPID was present in ~38% of the animals. Unsurprisingly then, both generalized and regionalized adiposity were evident in the cohort, with the findings of increased BCS and CNS consistent with the use of these phenotypic markers in the diagnosis of EMS,[10] and with a recent study that found increased adiposity to be a risk factor for endocrinopathic laminitis.[12] These data are important as recognition of an underlying endocrinopathy might enable the timely implementation of management (EMS) or treatment (PPID) strategies.

Concurrent inciting factors in the same animal were recognized in a reasonable proportion (~45%) of the cases. Recently, it has been suggested that laminitis occurring in animals with PPID is associated with basal (resting) hyperinsulinemia.[28] Although a small proportion (~10%) of animals in the current study was found to have PPID and a normal resting insulin concentration, it is worth considering that resting insulin concentrations fluctuate. Therefore, insulin dysregulation could still have been present in the animals with PPID and might have contributed to their laminitis. Other tests for insulin dysregulation, such as the OGT/OST or the combined glucose-insulin test, would have been helpful to further investigate these cases.[29] Further research on the relationship between PPID and EMS, and any effect on laminitis risk, is required. The finding that concurrent PPID and EMS in a horse/pony resulted in markedly higher basal insulin concentrations than those recorded in animals with a single underlying factor is an important outcome of the study, because higher insulin concentrations are likely to increase laminitis risk.[9,30] Given that insulin was also correlated with the Obel grade of lameness, these findings suggest that animals with higher insulin concentrations are also likely to suffer laminitis of greater severity, and as such, animals with both EMS and PPID require particularly judicious monitoring and management to prevent laminitis.

This study also found that serum insulin concentration was likely to be higher in smaller (shorter) animals. A negative correlation between serum insulin concentration and height at the withers has also been reported previously.[31] Differences in metabolism and insulin sensitivity

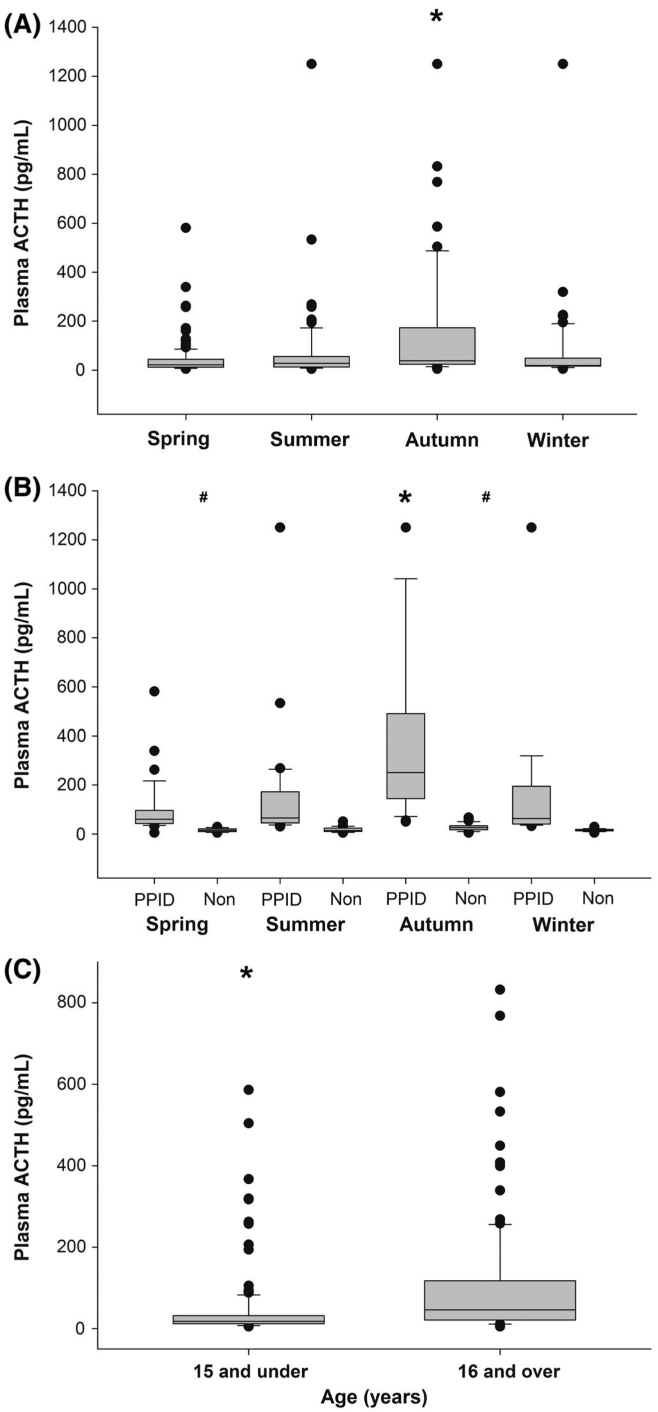

FIGURE 3 The plasma adrenocorticotropic hormone (ACTH) concentrations in a cohort of horses and ponies with endocrinopathic laminitis. The ACTH concentration was higher (**P* = .001) in autumn, compared to all other months, for the entire cohort (A). The ACTH concentration was higher (#*P* < .01) in autumn, compared to spring, in horses without PPID (B). Whereas in animals with PPID, the ACTH concentration in autumn was higher than in all other seasons (**P* < .01). Animals younger than 15 years of age had lower (**P* < .001) ACTH than animals aged 16 years and above (C)

have been demonstrated between horses and ponies,[32] and the data from the current study are supportive of the premise that ponies might be more likely to be insulin-dysregulated than insulin-sensitive breeds of horse, and therefore at an increased risk of laminitis. Genetic studies into the heritability of metabolic dysfunction, and laminitis risk, are still in their infancy, and an increased understanding in this area of equine medicine is greatly needed.[33]

In addressing its secondary aim, this study found no difference in the resting insulin concentrations of animals that were sampled before veterinary intervention, compared with those sampled after treatment/management was instigated. However, it was interesting to note that the median value for animals sampled at diagnosis was above the insulin cutoff for determination of EMS in this study, whereas the median value for animals sampled after intervention was below this cutoff. It is not known what factors could have been involved, but the instigation of dietary management or pain, or both of these, might have played a role. Therefore, we recommend that caution should be taken when interpreting the results of basal insulin measurements and that the conditions of sampling should be factored into this interpretation. Being cognizant of this, the clinician should also include an assessment of the animal's degree of pain at the time of sampling. Pain has been shown to adversely affect a number of clinical chemistry parameters, including insulin, in previous studies.[34,35] However, when considered in conjunction with the importance of measuring serum insulin in these animals, this finding does suggest that it is advisable for clinicians to measure insulin at the time the horse/pony is examined, rather than not at all.

Clinicians should also endeavor to educate horse/pony owners about the requirement for timely intervention for an animal with laminitis that might be in considerable pain, given the concerning finding from the current study that just over 50% of cases waited >10 days for veterinary attention. Recent reports that owners might fail to recognize laminitis in its early or mild stages is supportive of this perception.[8] However, it is also possible that farriery assistance was sought before veterinary intervention in some cases in the current study.

Insulin sensitivity appears to decline with age in horses.[36,37] Given that this also occurs in other species,[38,39] it is unknown why this outcome was not evident in the current study, but concurrent PPID in some cases could have been a confounding factor. However, basal/resting insulin concentration is an insensitive measure of insulin dysregulation in horses,[40] and it is probable that normal resting insulin concentrations were present in some animals with insulin dysregulation in the current study, which would have affected the ability to accurately assess the relationship between insulin sensitivity and age. By comparison, the positive association between age and ACTH concentration in the current study concurs with knowledge that PPID is a condition of aged animals.[41]

The significant effect of season on plasma ACTH concentration in the cohort of recruits is consistent with the current understanding that seasonal fluctuations of this hormone occur and confirms that the use of seasonally adjusted reference ranges is imperative.[21,42,43] The lack off an effect of sex and breed on plasma ACTH is interesting, given that a significant effect of both sex (mares) and breed (Shetland) has been reported previously.[42,44] Although that study was supported by a much larger sample size and thus had greater power to detect these effects,[44] the finding from our study that ACTH concentrations are higher in ponies (than horses) in autumn provides support for a potential seasonal effect on ACTH concentration in some breeds. Further research on the role of ACTH in laminitis onset, if indeed it has a direct role, is required.

The finding that the highest frequency of cases occurred during spring is consistent with a previous study undertaken in the United Kingdom that reported the highest incidence and prevalence of pasture-associated laminitis in May,[13] and also with a larger study undertaken in North America.[12] It could be conjectured that this increased incidence of laminitis in spring is related to either an increased availability of grass or an improved quality of grass at this time of year, where an increased NSC content of the grass can result in an increase in carbohydrate consumption.[45] Although in the current study basal insulin concentrations were not higher in spring, which would have supported this hypothesis, a postprandial insulin measurement might have been more informative. New access to pasture has been shown to increase an animal's risk of laminitis.[31] Access to pasture was a common feature of the housing arrangement for the vast majority of animals recruited to the current study and was consistent with the reasonably high frequency of pasture-associated reports of disease. However, it is important to note that the majority of cases with a pasture-associated factor also had an endocrinopathy. Taken together, these data suggest that some cases of endocrinopathic laminitis have a temporal pattern in individuals allowed unrestricted access to pasture, irrespective of the underlying endocrine disease, and further study on the relationship between pasture access and laminitis is required.

The clinical features of endocrinopathic laminitis recorded by the submitting veterinarians in this study were similar to those reported for other forms of the disease.[46,47] Similarly, the radiographic parameters reported were consistent with previous reports.[47,48] Thus, it appears that endocrinopathic laminitis might be indistinguishable from other forms of the disease (ie, the other inciting causes), when only clinical and radiographic examination findings from the foot are considered. However, it has been suggested that endocrinopathic laminitis is the most likely form to manifest as chronic disease, and the reasonably frequent reports of chronic change to the hoof in the current study, such as the presence of divergent growth rings, support that these are important features of the endocrinopathic form of laminitis.[49] Furthermore, the fact that just over 50% of the cases had experienced a previous episode of laminitis is supportive of the premise that this form of the disease has a reasonably high risk of recurring and becoming chronic.

Overall, this study has contributed to our understanding of equine endocrinopathic laminitis by showing that horses and ponies with concurrent endocrinopathies have more marked hyperinsulinemia and are therefore potentially at greater risk of suffering an episode of laminitis. Furthermore, by positively correlating basal insulin concentration with a measure of laminitis severity, this study has contributed to the knowledge that insulin concentration is integral to endocrinopathic laminitis, and that careful management of hyperinsulinemia should be of paramount importance when treating a horse/pony with endocrinopathic laminitis.

ACKNOWLEDGMENTS

The authors acknowledge the participants for their contributions to this study and James McGree for statistical advice. The data contained in the manuscript were presented in part at the Bain Fallon Equine Conference at the Gold Coast, Australia, 2017 and the Dorothy Havemeyer Foundation Equine Endocrinology Summit in Miami, 2017.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The study was approved by the Human and Animal Ethics Committees of Queensland University of Technology (1600000936, 1300000744).

HUMAN ETHICS APPROVAL DECLARATION

The study was approved by the Human and Animal Ethics Committees of Queensland University of Technology (1600000936, 1300000744).

ORCID

Melody A. de Laat https://orcid.org/0000-0001-7922-3642

REFERENCES

1. Pollitt CC. Equine laminitis. *Clin Tech Equine Pract*. 2004;3:34-44.
2. Karikoski NP, Horn I, McGowan TW, et al. The prevalence of endocrinopathic laminitis among horses presented for laminitis at a first-opinion/referral equine hospital. *Domest Anim Endocrinol*. 2011;41:111-117.
3. Donaldson MT, Jorgensen AJ, Beech J. Evaluation of suspected pituitary pars intermedia dysfunction in horses with laminitis. *J Am Vet Med Assoc*. 2004;224:1123-1127.
4. Patterson-Kane JC, Karikoski NP, McGowan CM. Paradigm shifts in understanding equine laminitis. *Vet J*. 2018;231:33-40.
5. Potter SJ, Bamford NJ, Harris PA, Bailey SR. Incidence of laminitis and survey of dietary and management practices in pleasure horses and ponies in South-Eastern Australia. *Aust Vet J*. 2017;95:370-374.
6. Menzies-Gow NJ, Harris PA, Elliott J. Prospective cohort study evaluating risk factors for the development of pasture-associated laminitis in the United Kingdom. *Equine Vet J*. 2017;49:300-306.
7. Wylie CE, Shaw DJ, Verheyen KL, et al. Decision-tree analysis of clinical data to aid diagnostic reasoning for equine laminitis: a cross-sectional study. *Vet Rec*. 2016;178:420.
8. Tadros EM, Fowlie JG, Refsal KR, et al. Association between hyperinsulinemia and laminitis severity at the time of pituitary pars intermedia dysfunction diagnosis. *Equine Vet J*. 2018;51:52-56.
9. Meier AD, de Laat MA, Reiche DB, et al. The oral glucose test predicts laminitis risk in ponies fed a diet high in nonstructural carbohydrates. *Domest Anim Endocrinol*. 2018;63:1-9.
10. Frank N, Geor RJ, Bailey SR, Durham AE, Johnson PJ, American College of Veterinary Internal Medicine. Equine metabolic syndrome. *J Vet Intern Med*. 2010;24:467-475.
11. Ireland JL, McGowan CM. Epidemiology of pituitary pars intermedia dysfunction: a systematic literature review of clinical presentation, disease prevalence and risk factors. *Vet J*. 2018;235:22-33.
12. Coleman MC, Belknap JK, Eades SC, et al. Case-control study of risk factors for pasture-and endocrinopathy-associated laminitis in North American horses. *J Am Vet Med Assoc*. 2018;253:470-478.

13. Menzies-Gow NJ, Katz LM, Barker KJ, et al. Epidemiological study of pasture-associated laminitis and concurrent risk factors in the south of England. *Vet Rec*. 2010;167:690-694.
14. Carter RA, Treiber KH, Geor RJ, et al. Prediction of incipient pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalised and localised obesity in a cohort of ponies. *Equine Vet J*. 2009;41:171-178.
15. Frank N. Insulin resistance in horses. *AAEP Proceedings*. 2006;52: 51-54.
16. de Laat MA, McGree JM, Sillence MN. Equine hyperinsulinemia: investigation of the enteroinsular axis during insulin dysregulation. *Am J Physiol Endocrinol Metab*. 2016;310:E61-E72.
17. Lindase S, Nostell K, Soder J, et al. Relationship between beta-cell response and insulin sensitivity in horses based on the oral sugar test and the euglycemic hyperinsulinemic clamp. *J Vet Intern Med*. 2017; 31:1541-1550.
18. Obel N. Studies on the Histopathology of Acute Laminitis [dissertation]. Uppsala, Sweden: Almqvist and Wiksells Boktryckeri AB; 1948.
19. Copas VE, Durham AE. Circannual variation in plasma adrenocorticotropic hormone concentrations in the UK in normal horses and ponies, and those with pituitary pars intermedia dysfunction. *Equine Vet J*. 2012;44:440-443.
20. Tinworth KD, Wynn PC, Harris PA, et al. Optimising the Siemens Coat-A-Count Radioimunnoassay to measure insulin in equine plasma. Paper presented at: Proceedings of the Equine Science Society Congress; 2009; Colorado.
21. Mc Gowan TW, Pinchbeck GP, Mc Gowan CM. 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 Vet J*. 2013;45:66-73.
22. Knowles EJ, Harris PA, Elliott J, Menzies-Gow NJ. Use of the oral sugar test in ponies when performed with or without prior fasting. *Equine Vet J*. 2017;49:519-524.
23. Borer KE, Bailey SR, Menzies-Gow NJ, Harris PA, Elliott J. Effect of feeding glucose, fructose, and inulin on blood glucose and insulin concentrations in normal ponies and those predisposed to laminitis. *J Anim Sci*. 2012;90:3003-3011.
24. Henneke DR, Potter GD, Kreider JL, et al. Relationship between condition score, physical measurements and body-fat percentage in mares. *Equine Vet J*. 1983;15:371-372.
25. Carter RA, Geor RJ, Staniar WB, et al. Apparent adiposity assessed by standardised scoring systems and morphometric measurements in horses and ponies. *Vet J*. 2009;179:204-210.
26. Wylie CE, Collins SN, Verheyen KL, et al. A cohort study of equine laminitis in Great Britain 2009-2011: estimation of disease frequency and description of clinical signs in 577 cases. *Equine Vet J*. 2013;45: 681-687.
27. McGowan CM. Endocrinopathic laminitis. *Vet Clin N Am-Equine*. 2010; 26:233-237.
28. Karikoski NP, Patterson-Kane JC, Singer ER, McFarlane D, McGowan CM. Lamellar pathology in horses with pituitary pars intermedia dysfunction. *Equine Vet J*. 2016;48:472-478.
29. Bertin FR, de Laat MA. The diagnosis of equine insulin dysregulation. *Equine Vet J*. 2017;49:570-576.
30. McGowan CM, Frost R, Pfeiffer DU, Neiger R. Serum insulin concentrations in horses with equine Cushing's syndrome: response to a cortisol inhibitor and prognostic value. *Equine Vet J*. 2004;36:295-298.
31. Wylie CE, Collins SN, Verheyen KLP, Newton JR. Risk factors for equine laminitis: a case-control study conducted in veterinary-registered horses and ponies in Great Britain between 2009 and 2011. *Vet J*. 2013;198: 57-69.
32. Bamford NJ, Potter SJ, Harris PA, Bailey SR. Breed differences in insulin sensitivity and insulinemic responses to oral glucose in horses and ponies of moderate body condition score. *Domest Anim Endocrinol*. 2014;47:101-107.
33. Treiber KH, Kronfeld DS, Hess TM, Byrd BM, Splan RK, Staniar WB. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. *J Am Vet Med Assoc*. 2006;228:1538-1545.
34. Seematter G, Binnert C, Martin JL, et al. Relationship between stress, inflammation and metabolism. *Curr Opin Clin Nutr Metab Care*. 2004;7: 169-173.
35. Sugimoto K, Rashid IB, Kojima K, et al. Time course of pain sensation in rat models of insulin resistance, type 2 diabetes, and exogenous hyperinsulinaemia. *Diabetes Metab Res Rev*. 2008;24:642-650.
36. Rapson JL, Schott HC 2nd, Nielsen BD, et al. Effects of age and diet on glucose and insulin dynamics in the horse. *Equine Vet J*. 2018;50:690-696.
37. Jacob SI, Geor RJ, Weber PSD, Harris PA, McCue ME. Effect of age and dietary carbohydrate profiles on glucose and insulin dynamics in horses. *Equine Vet J*. 2018;50:249-254.
38. Gulshan M, Yaku K, Okabe K, et al. Overexpression of Nmnat3 efficiently increases NAD and NGD levels and ameliorates age-associated insulin resistance. *Aging Cell*. 2018;17:e12798.
39. Garduno-Garcia JJ, Gastaldelli A, DeFronzo RA, et al. Older subjects with beta-cell dysfunction have an accentuated Incretin release. *J Clin Endocrinol Metab*. 2018;103:2613-2619.
40. Frank N, Tadros EM. Insulin dysregulation. *Equine Vet J*. 2014;46: 103-112.
41. McFarlane D. Equine pituitary pars Intermedia dysfunction. *Vet Clin N Am-Equine*. 2011;27:93-113.
42. McFarlane D, Paradis MR, Zimmel D, et al. The effect of geographic location, breed, and pituitary dysfunction on seasonal adrenocorticotropin and alpha-melanocyte-stimulating hormone plasma concentrations in horses. *J Vet Intern Med*. 2011;25:872-881.
43. Secombe CJ, Tan RHH, Perara DI, Byrne DP, Watts SP, Wearn JG. The effect of geographic location on circannual adrenocorticotropic hormone plasma concentrations in horses in Australia. *J Vet Intern Med*. 2017;31:1533-1540.
44. Durham AE, Shreeve S. Horse-factors influencing the seasonal increase in plasma ACTH secretion. In: International Equine Endocrinology Summit, Coral Gables, Florida; 2017;36–37.
45. Longland AC, Byrd BM. Pasture nonstructural carbohydrates and equine laminitis. *J Nutr*. 2006;136:2099S-2102S.
46. Belknap JK. Laminitis in horses. In: Musculoskeletal System. Merck Sharp & Dohme Corp 2016.
47. Pollitt CC. Equine laminitis: Current concepts. In: RIRDC, ed. *Barton ACT*, Australia: Rural Industries Research and Development Corporation, Horse RD&E Plan; 2008.
48. Sherlock C, Parks A. Radiographic and radiological assessment of laminitis. *Equine Vet Educ*. 2013;25:524-535.
49. Hunt RJ, Wharton RE. Clinical presentation, diagnosis, and prognosis of chronic laminitis in North America. *Vet Clin N Am-Equine*. 2010;26: 141-153.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: de Laat MA, Sillence MN, Reiche DB. Phenotypic, hormonal, and clinical characteristics of equine endocrinopathic laminitis. *J Vet Intern Med*. 2019;33: 1456–1463. https://doi.org/10.1111/jvim.15419