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The Effect of Geographic Location on Circannual Adrenocorticotropic Hormone Plasma Concentrations in Horses in Australia

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Background: Longitudinal evaluation of plasma endogenous ACTH concentration in clinically normal horses has not been investigated in the Southern Hemisphere.

Objectives: To longitudinally determine monthly upper reference limits for plasma ACTH in 2 disparate Australian geographic locations and to examine whether location affected the circannual rhythm of endogenous ACTH in the 2 groups of horses over a 12-month period.

Animals: Clinically normal horses <20 years of age from 4 properties (institutional herd and client owned animals) in Perth (n = 40) and Townsville (n = 41) were included in the study.

Methods: A prospective longitudinal descriptive study to determine the upper reference limit and confidence intervals for plasma ACTH in each geographic location using the ASVCP reference interval (RI) guidelines, for individual months and monthly groupings for 12 consecutive months.

Results: Plasma endogenous ACTH concentrations demonstrated a circannual rhythm. The increase in endogenous ACTH was not confined to the autumnal months but was associated with changes in photoperiod. During the quiescent period, plasma ACTH concentrations were lower, ≤43 pg/mL (upper limit of the 90% confidence interval (CI)) in horses from Perth and ≤67 pg/mL (upper limit of the 90% CI) in horses from Townsville, than at the acrophase, ≤94 pg/mL (upper limit of the 90% CI) in horses from Perth, ≤101 pg/mL (upper limit of the 90% CI) in horses from Townsville.

Conclusions and Clinical Importance: Circannual rhythms of endogenous ACTH concentrations vary between geographic locations, this could be due to changes in photoperiod or other unknown factors, and upper reference limits should be determined for specific locations.

Key words: ACTH; Horse; Photoperiod; Reference limit.

Measurement of endogenous ACTH in plasma is the most frequently used screening test to diagnose the common equine endocrinopathy pituitary pars intermedia dysfunction (PPID).[1,2] Seasonal variation of plasma concentration of this hormone in clinically normal horses and horses with PPID is well recognized, with higher autumnal plasma ACTH concentrations in both the Northern and Southern Hemispheres.[3–5] The variation in plasma ACTH concentration in healthy horses could be reflective of a circannual rhythm related to changing photoperiod.[6]

Abbreviations:

ASVCP	American Society of Veterinary Clinical Pathology
CI	confidence intervals
PPID	pituitary pars intermedia dysfunction
RI	reference interval

Upper reference limits of plasma ACTH concentration have been determined by examination of individual horses, with plasma ACTH measured at a single time point.[3,5] There are a number of studies that have longitudinally evaluated clinically normal horses over a 12-month period; however, these studies have not had sufficient sample size to generate an upper reference limit.[4,7–9]

The first objective of the study was to longitudinally determine monthly upper reference limits for plasma concentration of ACTH in 2 disparate locations within Australia. The second objective was to examine whether geographic location within Australia affected the circannual rhythm of plasma concentration of ACTH in the 2 groups of clinically normal horses over a 12-month period.

Materials and Methods

The upper reference limit for plasma ACTH was determined using the American Society of Veterinary Clinical Pathology (ASVCP) RI guidelines.[10]

Study Population

Horses were selected at each of 2 geographic locations within Australia; Perth in southern Australia (31°57′S, 115°52′E) and

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The work was undertaken in Australia in 2 locations: Perth, Western Australia and Townsville, Queensland.

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Townsville in northern Australia (19°26′S, 146°81E). The study population was recruited from 4 properties at each location. A thorough clinical examination was performed on all candidates, and horses were only included if they did not have any clinical signs consistent with PPID and were less than 20 years of age. Ethics approval was obtained from Murdoch University Animal Ethics Committee (Approval number R2702/14) and James Cook University Animal Ethics Committee (Approval number A2127).

Sample Collection

Sample collection began in March 2015 and ended in February 2016. Before each sample collection, all horses had physical examinations, including body condition scoring,[11] palpation of fore feet digital pulses, walking and turning in a tight circle in both directions. Any horse that had an increased digital pulse or was hesitant on turning had a hoof tester examination. Horses were sampled in their own environment, either in their paddocks or when they came into the yard for routine activities. Horses were all sampled in the morning (within 2 hours of each individual horse's previous sample collection) in the middle of each month. Blood was collected from the left jugular vein through a 20-g 1.5-inch vacutainer needle into evacuated 4-mL plastic tubes containing EDTA[a] and evacuated 4-mL plastic plain tubes.[b] The samples collected into tubes containing EDTA were placed on ice within 1 minute of collection and were centrifuged within 4 hours in a refrigerated centrifuge at 4000 × ***g*** for 5 minutes. Plasma and serum were aliquoted and placed into plastic vials[c] and stored in a −80°C freezer until batch sample processing 1–12 months later. Data on day length at each location were gathered from Geoscience Australia.[d] A subset of horses in each location had a TRH stimulation test as previously described, to further support case selection based on clinical normality.[12]

ACTH Analysis and Quality Control

Frozen plasma samples were transported on dry ice, and plasma ACTH concentrations analyzed using an Immulite 1000 ACTH solid-phase, 2-site sequential, chemiluminescent immunometric assay[e] performed on an Immulite 1000 analyzer[f] at IDEXX laboratories.[g] The analytical sensitivity of the assay is 9 pg/mL.[e] Quality control materials provided by the manufacturer were used daily (Immulite ACTH control module). The coefficients of variation for lower (29 pg/mL) and upper (414 pg/mL) level quality control materials were 4.16% and 5.07%, respectively, and were deemed acceptable.[h]

Statistical Analysis

Monthly data were tested for normality using the Shapiro-Wilk test and log-transformed if normality was not met. Outliers were identified by both the Tukey and the Reed method and were removed if determined to be aberrant observations. An aberrant observation was defined as a plasma ACTH value that was not comparable to the months preceding and after in that individual horse when all other monthly values for that horse were comparable to the rest of the group. Reference intervals and confidence intervals (CI) for plasma ACTH were calculated using parametric methodology by the software package *R*-studio[i] and robust methodology by MedCalc 16.4.3 software.[j] Statistical significance was assumed when $P \leq 0.05$.

Repeated measures ANOVA with Bonferroni's correction was used to determine differences in plasma ACTH over each individual month. When monthly means were not statistically different from each other, they were grouped and linear mixed modeling was used to determine the means and standard deviations to be representative of each group. Reference intervals were determined using the mean ± 2 standard deviations as the upper and lower reference limits, respectively, and 90% CI were obtained for the upper and lower reference limits.

Results

The final study cohort in Perth included 40 horses; the age range of horses was 4–15 years with an average and median age of these horses being 10.3 and 10.5 years, respectively. For 31 horses, samples were available for 12 months, with 9 horses having samples available over 11 months. Horse breeds were mixed and included Thoroughbreds (n = 11), Standardbreds (n = 12), Warmbloods (n = 3), mixed breeds (n = 6), and ponies (n = 8); there were 26 geldings and 14 mares. Thirty-eight horses were kept in paddocks and received supplemental feed of either roughage only or grain and roughage depending on their level of physical activity. Two horses were stabled at night and paddocked during the day for the majority of the study. All horses received routine anthelmintic care; vaccination status was unknown.

The final study cohort in Townsville included 41 horses, with an age range of 3–19 years; the average and median age of these horses was 11.6 years and 12 years, respectively. For 35 horses, samples were available for 12 months, with 6 horses having samples available over 11 months. There were 37 mares, 3 geldings, and 1 stallion. Breeds represented were Thoroughbreds (n = 23), Standardbreds (n = 7), Quarter Horses (n = 4), Australian Stock Horses (n = 6), and Warmblood horses (n = 1). All horses in the Townsville cohort were paddock housed and received supplemental feed of roughage only or grain and roughage depending on their level of physical activity. All horses received routine anthelmintic care and were vaccinated against tetanus and Hendra virus.

For the duration of the study, on intermittent occasions, individual horses in both cohorts had transient variations in foot related physical examination variables; however, no horse developed laminitis or other clinical signs consistent with PPID. For those individual horses that undertook some form of exercise in the preceding 24 hours, it was not deemed to be excessive. No horse was determined to be unwell on the day of sample collection.

Perth Data

Normality was not met, and monthly data were log-transformed to meet a Gaussian distribution. This was achieved for all months except July. Three outliers from different horses were removed from the data set; 2 from April (120 pg/mL and 138 pg/mL) and 1 from September (79.7 pg/mL). The outliers were considered to be unusual values as plasma ACTH in the months preceding and after were comparable to the rest of the group. The range and median plasma ACTH values (pg/mL) of each of these horses after outlier removal were 23.0–63.5 (31.8), 15.4–63.4 (28.4), and 23.3–62 (35.1), respectively. Additionally, 2 of the 3 horses with outlier values

had a TRH stimulation test performed and the plasma ACTH did not exceed concentrations after stimulation recommended by the equine endocrinology group.[13] Laboratory error in December resulted in 6 samples being excluded that month and 3 horses were unavailable for collection in January. Sample sizes for each month were 40 with the exception of September (n = 39), December (n = 34), January (n = 37), and April (n = 38). Summary of data is presented in Table 1 (median, maximum, and minimum values). Upper reference limits and 90% CI are presented in Table 2. Confidence interval width compared to reference limit width ranged from 0.33 to 0.49.

The repeated measures ANOVA with Bonferroni's correction suggested groupings of: June to November; December, January, and May; and February to April. Each of these groups was then analyzed using linear mixed modeling which more accurately accounts for between and within subject (horse) variation, to determine mean plasma ACTH concentrations to represent the groups and months within each group. For the first group, when June was compared with July ($P = 0.186$), August ($P = 0.468$), September ($P = 0.576$), October ($P = 0.312$), and November ($P = 0.065$), there was no significant difference in mean plasma ACTH concentrations. For the second grouping, when the month of January was compared to May there was no significant difference ($P = 0.552$); when compared to December ($P = 0.043$), the means were significantly different. For the third grouping, when the month of April was compared to March there was no significant difference ($P = 0.172$); when compared to February ($P = 0.014$), the means were significantly different. Although groups 2 and 3 did not achieve complete statistical homogeneity when analyzed using linear mixed modeling, they did so using repeated measures ANOVA and from a biological perspective these groupings were deemed valid. The upper reference limits and 90% CI were determined for each grouping (Table 3). Confidence interval width compared to reference limit width in the grouped data ranged from 0.14 to 0.22.

Table 1. Plasma ACTH concentrations from the raw data used to generate reference intervals before log transformation from the Perth cohort.

Month	Median ACTH concentration (pg/mL) (range), n
July	21.40 (15.30–44.50), 40
August	23.40 (10.30–41.40), 40
September	21.60 (12.40–33.70), 39
October	24.20 (13.80–38.70), 40
November	25.40 (16.00–44.30), 40
December	27.80 (16.50–53.30), 34
January	28.40 (19.20–59.60), 37
February	36.10 (19.60–70.30), 40
March	44.65 (23.00–80.50), 40
April	40.55 (28.50–67.60), 38
May	29.80 (16.30–68.00), 40
June	23.30 (14.80–49.50), 40

Table 2. Plasma ACTH upper reference values (90% CI) using parametric and robust methodology for the samples from horses in Perth.

	Plasma ACTH (pg/mL)	
Month	Parametric (90% CI)	Robust (90%CI)
July	*	34.37 (30.20–39.91)
August	42.30 (32.23–48.06)	41.89 (35.59–49.71)
September	35.65 (32.15–39.53)	36.37 (31.68–40.15)
October	37.72 (34.17–41.65)	38.56 (34.78–42.32)
November	41.68 (37.21–46.69)	41.46 (37.06–46.72)
December	48.48 (42.62–55.14)	46.72 (42.41–56.33)
January	53.86 (47.12–61.56)	56.33 (45.95–62.25)
February	68.70 (59.83–78.89)	69.93 (60.36–79.48)
March	81.97 (71.60–93.86)	83.20 (71.12–94.47)
April	65.38 (58.93–72.53)	64.82 (57.06–73.13)
May	54.70 (48.13–62.17)	53.89 (45.74–62.78)
June	40.21 (35.49–45.35)	40.38 (35.79–45.33)

*Data not normally distributed after log transformation therefore parametric analysis not applicable.

CI, Confidence interval.

Townsville Data

Normality was not met, and monthly data were log-transformed to meet a Gaussian distribution. This was achieved for all months except August, September, and December. Six outliers from different horses were removed from the data set; 1 each from November (185.0 pg/mL), December (104.0 pg/mL), and January (101.0 pg/mL), and 3 from February (101.0 pg/mL, 113.0 pg/mL, 200.0 pg/mL). The outliers were considered to be unusual values as plasma ACTH in the months preceding and after were comparable to the rest of the group. The range and median plasma ACTH values (pg/mL) of each of these horses after outlier removal were as follows: 41.90–88.10 (60.8), 31.20–60.80 (39.3), 25.4–63.9 (36.5), 23.6–87.5 (41.6) 29.5–89.9 (48.8), 14.4–43.4 (27.7), respectively. Additionally, 3 of the 6 horses with outlier values had a TRH stimulation test performed and the plasma ACTH did not exceed concentrations after stimulation recommended by the equine endocrinology group.[13] Laboratory error or horses being unavailable for collection resulted in 2 samples being excluded for the month of January, and 1 sample being excluded from August, September, October, and February. Sample size was 41 for March-July, 40 for August-December, 38 for January, and 37 for

Table 3. Plasma ACTH upper reference values with 90% CI using grouped data from horses sampled in Perth.

Month	Upper reference values (pg/mL) (90% CI)
June–November	41.12 (39.3–43.01)
May/December/January	52.51 (48.77–56.54)
February–April	78.18 (72.72–84.04)

CI, Confidence interval.

February. Summary of data is presented in Table 4 (median, maximum, and minimum values). Upper reference limit and 90% CI are presented in Table 5.

Table 4. Plasma ACTH concentrations from the raw data used to generate reference intervals before log transformation from the Townsville cohort.

Month	Median ACTH concentration (pg/mL) (range), n
July	30.60 (19.00–59.50), 41
August	33.25 (12.10–88.10), 40
September	30.30 (20.60–79.10), 40
October	23.35 (10.70–61.40), 40
November	24.00 (10.50–89.90), 40
December	29.95 (19.30–82.90), 40
January	34.95 (21.00–78.00), 38
February	40.90 (27.20–75.90), 37
March	48.60 (31.00–99.80), 41
April	34.70 (18.10–66.30), 41
May	34.30 (18.90–79.90), 41
June	29.70 (17.80–60.80), 41

Table 5. Plasma ACTH upper reference values (90% CI) using parametric and robust methodology for the samples from horses in Townsville.

	Plasma ACTH (pg/mL)	
Month	Parametric (90% CI)	Robust (90%CI)
July	*	50.76 (45.07–56.60)
August	62.66 (54.16–72.48)	62.25 (50.74–75.61)
September	*	60.34 (49.41–70.80)
October	45.42 (39.19–52.64)	44.40 (37.00–52.85)
November	55.38 (46.38–66.14)	56.48 (45.12–68.42)
December	*	45.69 (37.95–55.25)
January	76.55 (65.56–89.4)	77.21 (62.02–91.64)
February	75.59 (66.74–85.63)	76.35 (64.88–87.14)
March	88.90 (78.48–100.71)	88.87 (77.49–101.34)
April	73.09 (62.09–86.05)	73.38 (60.24–86.09)
May	68.58 (58.75–80.05)	67.18 (56.26–80.11)
June	49.94 (44.33–56.26)	49.45 (43.46–56.26)

*Data not normally distributed after log transformation therefore parametric analysis not applicable.

CI, Confidence interval.

Table 6. Plasma ACTH upper reference values with 90% CI using grouped data from horses sampled in Townsville.

Month	Upper reference values (pg/mL) (90% CI)
July–September, December, April–May	62.92 (59.55–66.49)
October	45.42 (39.19–52.64)
November	55.38 (46.38–66.14)
January	76.55 (65.56–89.40)
February	75.59 (66.74–85.63)
March	88.90 (78.48–100.71)
June	49.94 (44.33–56.26)

CI, Confidence interval.

Confidence interval width compared to reference limit width ranged from 0.34 to 0.45.

The repeated measures ANOVA with Bonferroni's correction suggested grouping as follows: April to September and December; June, October, and November; January and February; with March as a stand-alone month. Each of these groups was then analyzed using linear mixed modeling as for the Perth data. For the first group, when the month of August was compared with July ($P = 0.105$), September ($P = 0.637$), December ($P = 0.068$), April ($P = 0.129$), and May ($P = 0.308$), there was no significant difference in mean plasma ACTH concentrations. However, the month of June was significantly different ($P = 0.033$). For the second grouping suggested by the repeated measures ANOVA, June was significantly different in mean plasma ACTH concentrations to October ($P = 0.0002$) and November ($P = 0.02$). October was significantly different to November ($P = 0.03$). Hence, these months were treated as stand-alone months in the analysis. For the third grouping, February was significantly different to January ($P = 0.009$). As a result, January, February, and March are all treated as stand-alone months (Table 6). Confidence interval width compared to reference value width in the grouped data inclusive of the individual months ranged from 0.16 to 0.45.

Day Length

The shortest day of the year (winter solstice) occurred on June 22, 2015 and the longest day of the year (summer solstice) on December 22, 2015. The equinox dates for 2015 were March 21 and September 23. Day length changes in comparison with plasma ACTH concentrations at each location are presented in Figure 1.

Discussion

This study generated individual normal upper reference limits for plasma ACTH concentration for each month of the year and specific monthly groupings in 2 geographic locations within the Southern Hemisphere. This study differs from previous studies in that sufficient individual clinically normal horses were longitudinal tracked monthly over a 12-month period to generate monthly upper reference limits and confidence intervals. Endogenous ACTH in this study had a circannual rhythm, consistent with other studies.[3–5,7–9,14–16] The increase in plasma ACTH was not confined to only the autumnal months as has been previously described[3–5,8,9] but was consistent with the change in photoperiod as has been described by Durham.[6]

The generation of veterinary reference limits should follow specific guidelines, and within this study, the ASVCP guidelines[10] were used. Although it was the intention to utilize a sample size of 40 horses for each month, this was not possible. As all sample sizes were greater than 20 and as plasma ACTH concentrations of most months were considered Gaussian in their distribution after transformation, both robust and parametric methodologies were performed to determine the

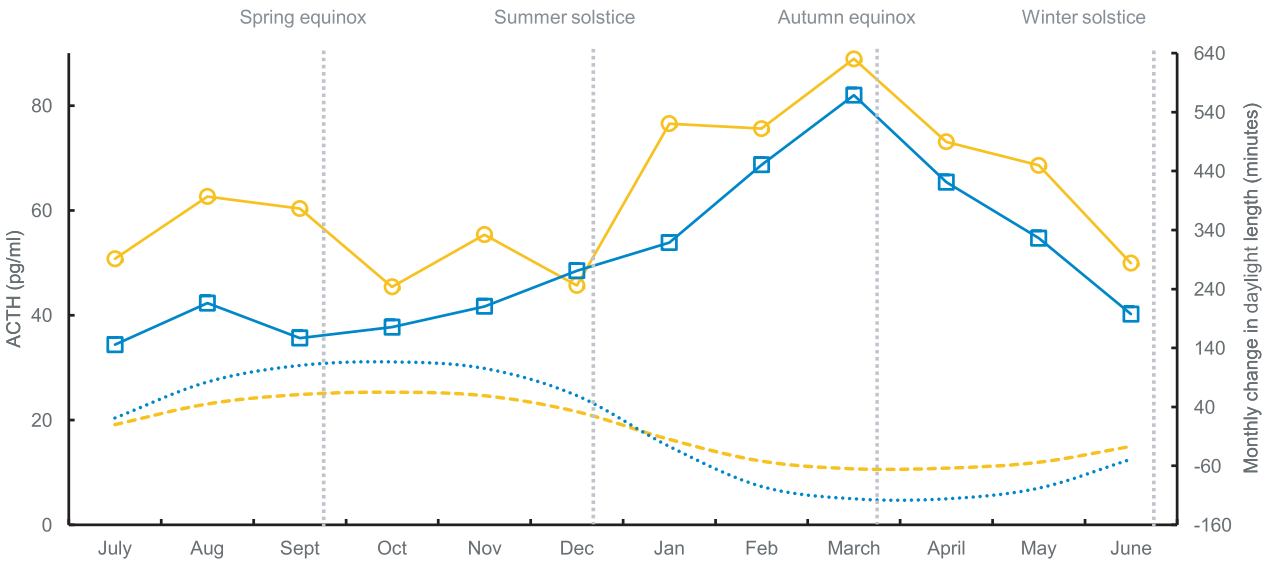

Fig 1. Circannual plasma ACTH upper reference values (Townsville —○—, Perth —□—) and day length change (Townsville ·····, Perth - - - -). Parametric with robust values for months with non-normal data distribution. [Color figure can be viewed at wileyonlinelibrary.com]

reference limits and median, maximum, and minimum plasma ACTH values are presented (Tables 1 and 4). Two methodologies were employed as similarity in RI, as was demonstrated in this case, supports a homogenous population.[17]

Reference intervals are essential to assist a clinician in making a clinical decision regarding the presence or absence of disease. Reference limits only describe the variability of the measured analyte within a population of normal animals and the value at which the analyte should be considered normal in the general population is not below the upper reference limit, but can be considered to be below the upper limit of the CI.[18] Confidence intervals were calculated for all months by both the parametric and robust methodologies and were found to be similar (Tables 2 and 5). The CI widths compared to the width of the RI for all months in both data sets exceeded 0.2; the recommendation suggested by the ASVCP.[10] This is not surprising given these criteria cannot be met with a sample size of less than 55.[18] When monthly data were grouped, the CI widths compared to the width of the RI were ≤ 0.2. When assessing analytes where there is a pronounced difference between health and disease, as has been shown in particular with autumnal plasma ACTH concentrations in horses with clinical signs of PPID,[3,5,8] higher imprecision around the upper reference limit should not affect clinical interpretation.[18]

Two distinct periods were identified within the circannual rhythm of endogenous ACTH. A period when the upper reference limit for plasma ACTH was lower compared to the rest of the year with little variability and is defined by the authors as the "quiescent phase." This was June to November (6 months) inclusive in Perth as these months were statistically homogenous. In Townsville, the quiescent period might be from April to December (9 months) as suggested by statistical analysis; however, it is possible it follows a similar pattern to Perth (Fig 1), which only might become evident with larger sample sizes or investigation with other endocrine tests such as monthly TRH stimulation testing. A second period commences when plasma ACTH begins to increase, reaches a peak (acrophase), and then declines, this period has been defined by the authors as the "dynamic phase." The upper reference limits during this phase were higher and more variable. Geographic location appeared to influence the length of the dynamic ACTH phase with a potentially longer dynamic phase the further south the location. In both locations, the plasma ACTH upper reference limit was similar at the acrophase (March in both locations) and coincided with the autumn equinox. The authors surmised the greater the change in circannual photoperiod the lower the upper reference limit of plasma ACTH during the quiescent phase with a potentially longer dynamic phase of ACTH. This is supported by the upper reference limit of plasma ACTH in Perth being lower than in Townsville during the quiescent period. If one of the roles of ACTH is to alter equine metabolism in preparation for winter, it seems reasonable to assume that less circannual fluctuation would occur in tropical equatorial regions that have an absence of true "seasons." Further research would need to be undertaken to determine whether in addition to photoperiod factors such as climate have a role in circannual rhythm of ACTH.

Due to the monthly variation during the dynamic phase, grouping of the data was problematic. In Perth, 2 of the groupings chosen, February to April inclusive and December, January, and May did not display complete statistical homogeneity within the groups; however, these 2 groupings were maintained from a clinical

decision-making perspective as there was little difference in the upper reference limits suggesting biological similarity. It is up to the individual clinician to determine whether they wish to use a monthly CI upper limit or a grouped CI upper limit. In Townsville, plasma ACTH concentrations during the dynamic phase did not display any statistical homogeneity and as such clinicians are recommended to use monthly CI upper limits for that period. Plasma ACTH concentrations were lower in June, October, and November within the proposed quiescent period of April to December; the clinical relevance of this is unknown; hence, individual clinicians should determine whether they wish to use the higher CI upper limit for the grouped months (July, August, September, December, April, and May) from April to December inclusive or use monthly CI upper limits.

Although a cutoff value of plasma ACTH in clinically normal horses for a latitude between the 2 locations of this study has been reported,[5] samples were collected from horses at a single time point, data were grouped into seasons, and a differing statistical methodology was used; therefore, it is difficult to compare the 2 studies. Within that study,[5] the cutoff value for both the autumnal and nonautumnal periods was lower than either the Perth or Townsville limit of the upper CI. The authors are of the opinion that this is not due to case selection criteria as this study used younger animals with no clinical signs of PPID where disease prevalence is lower, rather it is likely to be due to that study's use of an alternate statistical methodology.

Although the limit of the upper CI for plasma ACTH during the dynamic phase in both Australian locations was similar to what has been reported in the Northern Hemisphere,[13] the limit of the upper CI for the quiescent phase was higher compared to the Northern Hemisphere. This might be due to study design or the probability that local geographic and management factors have a combined effect with photoperiod in determining regulation of ACTH secretion. It must be acknowledged that if plasma ACTH concentrations exceed the limit of the upper CI, this does not necessarily confirm that the horse has PPID. Clinical decision limits, that is the value at which it is likely the animal has the disease, differ from reference limits and are usually based on guidelines reached from a group consensus of expert individuals.[19] With regard to PPID, guidelines regarding clinical decision limits continue to be reviewed, particularly in the Southern Hemisphere, as further research is conducted.

The selection criteria used to choose horses were similar to previous studies with both age and the absence of clinical signs associated with PPID guiding the inclusion of horses.[3,5,20] The inadvertent inclusion of horses with subclinical PPID was thought to be unlikely in this study as a subset of horses had TRH stimulation tests during the quiescent ACTH phase and were determined to be normal (unpublished data). In addition, prevalence of the disease is low in younger horses[21,22] and horses were sampled on multiple occasions so that if persistently increased levels were detected suggestive of PPID they could be excluded. Age does not affect plasma ACTH concentration in healthy horses[23] and as such the benefits of using a younger population with a lower prevalence of disease were thought to outweigh the risk of inadvertent inclusion of horses with subclinical disease had an older population been used.

Similar to other studies determining the upper limit of plasma ACTH[3,5] or the effect of location,[20] sex was not standardized across locations as sex does not affect plasma ACTH in equids.[14] Horses were only sampled at one time point on collection days as paired or multiple samples have not been shown to confer a benefit over a single measurement.[24,25] Although the literature is conflicting as to whether endogenous ACTH in horses has a well-defined circadian rhythm, all samples were collected within the same 2 hour time period each month to ensure consistency.[15,16,25]

On several occasions, isolated unusual plasma ACTH increases were detected that exceeded the upper reference limit for that time of year, predominantly during the ACTH dynamic phase when increased variability was appreciated. Within this study, an explanation as to why the isolated unusual increases occurred could not be provided as the horses were in good health and had not been subjected to perceivable physiological stress, which has been implicated in increasing plasma ACTH concentrations in horses.[26,27] These findings support the notion that if a single plasma ACTH test is being used to screen for PPID, interpretation should be taken in light of clinical presentation and repeat testing or performing a TRH stimulation test might be preferable over initiation of treatment.

Limitations of this study are that horses were only evaluated longitudinally for 12 months and the assumption is made that the 12-month period chosen is representative of the circannual rhythm; however, it is unknown whether this is the case. Secondly as this was not a terminal study, it is unknown whether these horses had subclinical PPID; however, this limitation is shared with many other studies evaluating plasma ACTH in clinically normal horses. Finally, ideally the horses would have been breed matched across each location with only 1 breed utilized as although previous work has suggested that there is no difference in plasma ACTH between ponies and horses,[5,14,20] emerging literature[28] suggests ACTH might differ between breeds.

In conclusion, this study achieved the aims of determining monthly RIs for 2 disparate locations within Australia. We demonstrated that the circannual rhythm is characterized by a quiescent phase where the upper reference limit of plasma ACTH is lower and variable between locations, but higher than those currently reported for the Northern Hemisphere. In comparison, there is a dynamic ACTH phase which results in a similar acrophase at the autumn equinox in both locations and the Northern Hemisphere. In Australia at locations closer to the equator, horses might have a shorter dynamic phase to their circannual ACTH rhythm with higher reference limits during the quiescent phase.

Footnotes

[a] Greiner Bio-One 4 mL plastic whole blood tube with spray coated K2EDTA, reference number 454023, Germany
[b] Greiner Bio-One 4 mL plastic whole blood tube with serum clot activator, reference number 454204, Germany
[c] Cryo.S® cryovials, Greiner Bio-One GmbH, Germany
[d] Geo Science Australia http://www.ga.gov.au/
[e] Siemens Immulite/Immulite 1000ACTH assay, Australia
[f] Siemens Immulite/Immulite ACTH control module, Australia
[g] IDEXX Laboratories Pty Ltd, Unit 6, 38 – 46 South St, Rydalmere NSW 2116 Australia
[h] The Royal College of Pathologists of Australasia and the Australasian Clinical Biochemist Association Quality Assurance Program
[i] R Studio Team (2015). R Studio: Integrated Development for R. R Studio, Inc., Boston, MA URL http://www.rstudio.com/
[j] MedCalc Software bvba, Mariakerke, Belgium; https://www.medcalc.org; 2016

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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