**Letter to the Editor**

**Recommendations from the Australian and New Zealand Equine Endocrine Group and the interpretation of plasma endogenous ACTH concentrations for the diagnosis of pituitary pars intermedia dysfunction (PPID).**

Dear Dr Jackson,

I read with interest the new open access publication entitled “*Equine pituitary pars intermedia dysfunction: current understanding and recommendations from the Australian and New Zealand Equine Endocrine Group”* 1 that was published online on June 3rd 2018. The authors are to be congratulated on providing an Australian slant on the recent Equine Endocrinology Group2 recommendations and some recent data from Australia.3 The paper is well written and provides valuable interpretation from leading endocrinology researchers and clinicians.

However, on reading it I was surprised to see new recommendations for interpretation of ACTH for the diagnosis PPID in Table 1. that did not appear evidenced by previous publications, including the 3 peer reviewed publications the authors reference to justify the recommendations.3,4.5 In particular, the work based on horses in Queensland, Australia.4 There appears to have been an inadvertent increase in the cut off values for PPID by adding a grey zone, not based on scientific evidence, nor usual laboratory practice. The key issues are as follows:

1. The grey zones are skewed by only starting from the high end of variation of normal (Fig. 1).3
2. The grey zones appear to have been extrapolated from normal horses,3 not horses with PPID
3. Published research which has included both normal and PPID affected Australian horses4 has not been appropriately factored into new recommendations for Australia.

For simplicity and brevity I will outline my concerns using the non-Autumn periods, but similar applies to the autumn values. I will also not cover the data from Northern Queensland. I am also assuming the same test (chemiluminescent assay/Immulite 1000/2000).

On reviewing the key literature around the interpretation of ACTH referred to in the paper, I can see how these grey zones were extrapolated.

Northern hemisphere key research (normal variation):

In a study based in southern UK published in 2012,5 156 clinically normal horses (4-28 years old) were used to determine an upper 97.5% reference limit and then a 95% CI calculation on that. The upper 95%CI was taken as a cut off value and this was 29 pg/ml in non-Autumn.

Although no references are provided, it appears that this derived cut off5 formed at least part of the basis for the extrapolation of an equivocal zone of 30-50 pg/ml for interpretation of ACTH by the USA based equine endocrinology group.2 They recommended that ACTH values from horses with suspected PPID in this zone should prompt use of a dynamic test for diagnosis of PPID.2

Southern hemisphere key research (normal variation):

In a study based in Perth, published in 20173, 40 clinically normal horses (4-15 years old) were sampled monthly for a year. The authors used a very similar methodology to the UK study5 to determine cut offs for ACTH. They derived an upper reference limit (2 standard deviations [SD]) and then performed a 90% CI calculation on that. The upper 90%CI of the upper 2 SD of the mean was taken as the limit and this was 43 pg/ml in the non-autumn quiescent period3, although varied month to month throughout the non-autumn period.

In both this paper3 and that performed in the UK5 the authors have attempted to use the furthest limit of the variation of normal to define a laboratory reference range i.e. the right side of a normal distribution pattern or bell curve of normal horses. (Fig. 1).

Three questions are raised by this; a. how do we know the horses were normal?; b. are younger controls appropriate? And, most importantly, c. there is no comparison with a diseased (PPID) group.

The next problem is that the endocrinology group1 have added a grey zone above this upper limit in an apparently similar way to the group from the USA2 (Figure 2.).

To add a grey zone above the upper 90% CI of the upper reference limit can only assume one (or both) of two things:

1. There is no overlap between diseased and normal or that the overlap is negligibly small (e.g. figure 3).

2. That actually diagnosing the disease (sensitivity) is not important and exclusion of false positives (specificity) is the primary clinical aim (which has welfare implications as concluded below).

Figures 3 and 4. illustrate schematically the potential effect of a grey zone depending on how close the distribution curves for normal and PPID affected horses are.

What about the diseased (PPID) group?

For the northern hemisphere there are limited data looking at PPID versus controls, but for the Southern hemisphere, the authors appear to have dismissed the findings of a paper from Queensland, Australia4 which is the only epidemiological study that allowed the used of advanced receiver operator characteristics (ROC) analysis (Youden Index analysis) due to the defining of a clinical gold standard PPID positive group and having an appropriate population prevalence estimate.4 In that paper they represented Youden index derived cut offs obtained from using an epidemiological sample of 325 horses aged 15 years and older between Bundaberg and the Gold coast, which after inclusions and exclusions were applied, left 217 clearly defined normal and 20 gold standard defined PPID positive cases.

The cut off of 29.7 pg/ml with 80% sensitivity and 83% specificity in the non-autumn period4 was below the lower limit of the grey zone (40-70 or 80 pg/ml) published in the new recommendations.1 This is the most robust data we have to date for the optimal cut off for ACTH in horses, represented schematically in figure 5 below.

In conclusion, by not appropriately factoring in robust evidence which has included both normal and PPID horses from the same age group, 4 the Australian endocrinology group have recommended reference ranges for interpretation of ACTH values that would provide poor sensitivity for the diagnosis of PPID. While undoubtedly highly specific, poor sensitivity may lead to failure to confirm a diagnosis in horses with PPID which has the potential to delay appropriate treatment potentially leading to a serious risk to the welfare of the horses concerned.

Catherine McGowan

Prof. Catherine McGowan BVSc,MACVSc,DEIM,Dip ECEIM,PhD,FHEA,MRCVS

RCVS and European Specialist in Equine Internal Medicine

Head of Department, Equine Clinical Science

Director of Veterinary Postgraduate Education

Institutes of Ageing and Chronic Disease and Veterinary Science,

Faculty of Health and Life Sciences

University of Liverpool

Leahurst, CH64 7TE, UK

C.M.Mcgowan@liverpool.ac.uk

**References**

1. Secombe CJ Bailey SR de Laat MA Hughes KJ Stewart AS Sonis JM Tan RHH (2018). Equine pituitary pars intermedia dysfunction: current understanding and recommendations from the Australian and New Zealand Equine Endocrine Group by Australian Veterinary Journal First published: 03 June.
2. Equine Endocrinology Group Recommendations for the Diagnosis and Treatment of Pituitary Pars Intermedia Dysfunction (PPID) Revised June 2017; Available from https://sites.tufts.edu/equineendogroup/files/2017/11/2017-EEG-Recommendations-PPID.pdf
3. Secombe CJ, Tan RHH, Perara DI, et al. (2017) The Effect of Geographic Location on Circannual Adrenocorticotropic Hormone Plasma Concentrations in Horses in Australia. J Vet Intern Med.;31(5):1533-1540.
4. McGowan TW, Pinchbeck GP, McGowan CM. (2013) Evaluation of basal plasma α-melanocyte-stimulating hormone and adrenocorticotrophic hormone concentrations for the diagnosis of pituitary pars intermedia dysfunction from a population of aged horses. Equine Vet J.;45(1):66-73.
5. Copas, V.E. and Durham, A.E. (2012) 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. 44, 440-443.

**Figure Legends**

Fig. 1 Schematic of the derived cut off values using normal horses based on the upper end of a 90% confidence interval (CI) around the upper end of two standard deviations (SD) from the mean.



Fig. 2 Schematic of an extrapolated grey zone from derived values in Fig. A.



Fig. 3 Schematic normal distribution curves for normal and pituitary pars intermedia (PPID) affected horses showing curves far apart from each other and the effect of derived cut offs and grey zones from normal horses.



Fig. 4 Schematic normal distribution curves for normal and PPID affected horses showing curves in a more realistic relationship showing some overlap. The use of derived cut offs and grey zones from normal horses becomes more profound meaning many horses with PPID could potentially be falsely diagnosed as negative.



Fig. 5 The same schematic as Fig. D showing where an optimal cut off should lie to maximise both sensitivity and specificity of a diagnostic test.

