**Equine Cushing’s Disease/PPID – what do we know now?**

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*Updates on pathophysiology and epidemiology of PPID and current best practice in diagnosis of PPID.*

**Introduction**

The importance of equine pituitary pars intermedia dysfunction (PPID or Equine Cushing’s disease) has become better understood in the last decade following some excellent pathophysiological research from the USA and Australian epidemiological research highlighting just how prevalent this disease is. The same group highlighted the disparity between owner recognition of the subtle signs of the condition and the actual prevalence found on blood tests. Although there have been many tests used to diagnose PPID in the past, testing practices are becoming more standardised with a better understanding of which test to use and when.

The aims of this presentation are to:

1: Recap the pathophysiology of PPID and evidence for it being a neurodegenerative disorder associated with aging in horses.

2: Discuss the epidemiology of PPID including its prevalence, risk factors, associated clinical signs and clinicopathological abnormalities, particularly laminitis and insulin dysregulation. Also early and more subtle clinical signs that owners may confuse with signs of old age.

3: Review current best practice in diagnosis of PPID including basal and dynamic tests, ways to improve early detection of PPID and the effect of season and making season work for you.

**Pathophysiology of PPID**

PPID is a common neurodegenerative disorder causing a loss of dopaminergic inhibitory control of the pars intermedia of the pituitary gland. It results from a loss of the inhibitory dopaminergic neurones in the hypothalamus and associated decreased dopamine and dopamine metabolites. The loss of dopaminergic neurons is associated with increases in the oxidative stress marker, 3-nitrotyrosine which is increased in normal aged horses (around 7X) but markedly increased in horses with PPID (16X) supporting that this is an age related neurodegeneration similar to Parkinson’s disease in people. 1

The result is a loss of control of pars intermedia endocrine function and the overproduction of proopiomelanocortin (POMC) derived peptides produced by the pars intermedia melonotrope cells including adrenocorticotropin (ACTH), alpha melanocyte stimulating hormone (α-MSH), beta endorphin (β-endorphin) and corticotrophin-like intermediate peptide (CLIP). Although a useful marker for diagnosis of PPID, much of the ACTH is biologically inactive and PPID is not associated with hyperadrenocortiscism as it is in dogs and people. This overactive pars intermedia becomes hyperplastic on histological examination initially, and in later stages can become adenomatous, although it is not a primary tumour as many owners may assume. 2,3

Although we still know relatively little about many of the peptides released by the abnormal pituitary pars intermedia and their clinical consequences, it is important to convey an appropriate understanding of the pathophysiology of PPID to horse owners so that they can understand the potential for medical treatment and monitoring of the disease.

**Epidemiology of PPID**

The importance of equine PPID has been highlighted by Australian research reporting a disease prevalence of 21.2% in a cross-sectional study of client-owned horses and ponies aged ≥15 years in Australia. 4 The horses were diagnosed based on elevated basal ACTH using seasonally adjusted reference ranges, 5 and age was the most important risk factor with the odds of having PPID increasing by 18% each year from 15 years of age. PPID has also been recognised in younger horses, however reports in horses <10 years old are very rare. Ponies were traditionally thought to have an increased susceptibility, but this was found to be a combination of greater longevity and more obvious hypertrichosis. Research has shown no effect of breed, 4 and it appears that all breeds of horse should be considered at risk of PPID.

Trends in horse ownership and horses presented for veterinary care are changing and aged horses are more frequently presented for veterinary care. They often highly experienced and valued by their owners as companion or teacher, and their value should not be underestimated. Despite this, many horse owners fail to recognise the clinical signs of PPID. In same study where veterinary examination revealed the prevalence of 21.2%, only 1.6% of horse owners reported their horses with a history of or current PPID (or synonym). 4 One reason for this may be that fact that epidemiological research has shown that many of the clinical signs of PPID are considered normal signs of aging by horse owners, including hypertrichosis and muscle atrophy. 6

*Clinical signs*

PPID presents with a variable combination of clinical signs, with the most specific clinical sign being a long hair coat (hypertrichosis) and/or delayed shedding, although in the early stages and in light breed horses this may be relatively subtle and not picked up by horse owners, or erroneously assumed to be a normal part of aging. One of the most common early signs is muscle atrophy presenting as a wasted topline and/or a pot belly occurring in 48% of horses 4, again poorly recognised by horse owners as a potential sign of disease. 6 The presentation of horses with PPID for veterinary attention requires the recognition of the clinical signs of PPID by their owners, so many cases are not presented until they have relatively advanced disease. This may be disease associated with PPID such as laminitis or secondary infections or diseases associated with being in the geriatric age bracket particularly dental disease (including periodontal disease), lameness, eye conditions, heart or lung conditions and skin conditions (including tumours such as sarcoids and melanomas). 7

The knowledge of the increased susceptibility aged and geriatric horses have to conditions and diseases as well as the bias of owner recognition are important to understand which diseases are associated with PPID and which are concurrent, as treatment failure may occur if concurrent diseases are ignored or assumed to be directly associated with PPID. An example are alterations in blood tests which have been reported as associated with PPID, 8 yet these have only been found in case series and not in appropriately controlled field based epidemiological research.4 PPID may increase the risk of intestinal parasitism but does not alter a routine haematological or biochemical profile.

Laminitis is an important and sometimes devastating clinical sign and horses with PPID have 4.65 times the odds of developing laminitis compared to aged matched controls. 4 It is important to recognise that hoof capsule changes associated with lamellar lesions can occur well in advance of lameness. 9 Owners will sometimes report previous episodes of laminitis, or there might be hoof (prominent/divergent growth rings, dropped sole, white line separation) or radiographic (remodelling/rotation of P3) changes consistent with previous or subclinical laminitis. Recurrent white line abscesses can complicate the clinical picture. Laminitis usually represents more advanced PPID and indicates concurrent insulin dysregulation.

Development of localised fat pads can occur with PPID, most commonly in the supraorbital fossae (bulging supraorbital fat. The fat “accumulation” in other parts of the body such as over the tail head, in the crest or rump is associated with underlying muscle atrophy or an effect of PPID is unclear.

Excessive sweating is sometimes a result of a long coat, however some horses continue to sweat excessively even when clipped. In more Northern areas of Australia anhidrosis has been reported which can cause heat stress and marked exercise intolerance. 10

Other clinical signs include lethargy, presumed to be caused by elevated β-endorphin concentrations and poor performance associated with muscle atrophy or other effects such as secondary infections or heat stress. β-endorphin might also have an analgesic effect in PPID, alleviating signs associated with painful conditions such as osteoarthritis. Polydypsia/polyuria which may be due to reduced action of antidiuretic hormone, increased thirst, or osmotic diuresis from hyperglycaemia. Increased susceptibility to infections and infertility (which may be related) and occasionally seizure like activity have been reported. Although it may seem logical that an abnormal pituitary gland might cause forebrain disease, the location of the pituitary is ventral associated with the brainstem not forebrain, and the only documented neurological effect has been blindness from a large adenoma affecting the optic chiasm.

**Diagnosis of PPID**

Two shifts in the approach to PPID diagnosis have occurred recently:

1. Earlier detection. PPID was once a condition that was diagnosed in response to relatively advanced clinical signs, often including recurrent laminitis. Advances in vets’ and clients’ understanding and awareness of PPID have resulted in earlier detection, allowing implementation of preventive strategies to avoid the more serious consequences such as laminitis. Detection of earlier cases of PPID does present difficulties as the clinical signs are more subtle, and laboratory abnormalities less pronounced.
2. Routine assessment of insulin status. The association between PPID and laminitis is likely to involve insulin dysregulation, 9 and yet not all horses with PPID are insulin dysregulated. 4 Assessment and monitoring of insulin status in horse with PPID is essential to ensure management is adjusted appropriately to resolve ID and reduce the risk of laminitis.

**Laboratory testing of PPID**

Laboratory testing for PPID is not straight forward, with marked seasonal fluctuations in pituitary function and varying reference ranges in different publications. Several tests are described, and although results are usually interpreted using cut-offs as ‘positive’ or ‘negative’, it is more useful to regard them as representing a spectrum of severity of disease.

*Basal Plasma ACTH*

This is a convenient and by far the most commonly performed diagnostic test for PPID. It can be performed as a stand-alone test, or as part of the TRH stimulation test (see below). Samples can be collected at any time of day.

Protocol:

1. Collect blood into EDTA tube.
2. Chill the sample as soon as possible (within 3 hours of collection)
3. Separate the plasma prior to shipping/storage
4. Chill (don’t freeze unless centrifuged) during shipping to laboratory

Interpretation

There is a seasonal increase in ACTH concentration in autumn in both normal and PPID horses. The rise is greater in PPID horses, meaning that ACTH is actually a more sensitive and specific test during autumn, as long as appropriate seasonal adjustment of reference ranges is made.5

The reference range used depends on the laboratory and assay being used. More recently there have been data published from Australia refining ACTH cut off values specific to each month of the year from different geographical locations. 11 These data were from younger horses with no identified clinical signs of PPID and supported a higher upper reference range limit than the cut offs derived for an aged population of horses ≥ 15 years using a clinical gold standard of hypertrichosis and 3 clinical signs of PPID. 5

*TRH Stimulation Test*

Administration of TRH causes an increase in ACTH and other pituitary hormones that is greater in horses with PPID, peaks after 2-10 minutes and returns towards baseline after 30-60 minutes. There is a strong seasonal influence on the TRH stimulation test, with 10 minute ACTH concentrations increasing markedly in autumn in both PPID and non-PPID horses.

The TRH stimulation test has higher sensitivity compared to basal ACTH in the non-Autumn period, so is useful in early/subtle cases of PPID or as a second line test after borderline ACTH results.

Protocol:

1. No concentrate feeding for 3-4 hrs before
2. Collect EDTA sample for basal ACTH measurement
3. Inject 1mg\* of TRH IV
4. Collect further EDTA sample exactly 10 minutes after injection
5. Handle EDTA samples for ACTH as described above

\*The dosage of TRH is the same regardless of the size of the horse. Administering 50-200% of the dose has no effect on diagnostic outcome.

TRH is an unlicensed drug in horses. Adverse effects of TRH are commonly seen shortly after administration, including yawning, chewing, flehmen response and muscle fasciculations.

A cut-off of 110 pg/ml has been recommended for the 10-minute post-TRH, ACTH concentration. Until recently, testing in autumn was not advised based on a lack of reference ranges. Recent data suggest that the non-autumn cut offs should be higher than 110pg/ml, and that autumn testing is diagnostic, but with a much higher cut-off. 12

*Overnight dexamethasone suppression test (ODST)*

In normal horses nearly all plasma ACTH is secreted by the *pars distalis*, which is subject to negative feedback from endogenous cortisol and exogenous corticosteroids. In PPID horses the *pars intermedia* produces a significant amount of plasma ACTH, but is not under the same negative feedback, meaning that ACTH and cortisol concentrations are maintained. The ODST was once considered the gold standard antemortem test for PPID, but has fallen out of favour as there are concerns about administering dexamethasone to horses already at increased risk of laminitis, it requires 2 visits, and the specificity in autumn is poor.

Protocol:

1. Collect baseline sample for cortisol (4-6pm)
2. Inject 0.04mg/kg dexamethasone i/m
3. Collect a second sample for cortisol 19-20hrs later
4. Centrifuge sample prior to shipping

Interpretation

Using a cut-off of 27nmol/L the ODST is reported to have a sensitivity of 89% and specificity of 88%. These figures were derived from studies in which horses were selected with overt signs of PPID though, enhancing the performance of the test. For earlier/more subtle cases the ODST is likely to lack sensitivity compared to basal ACTH or TRH stimulation.

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