Research priorities, horse owner perceptions and practices regarding management and treatment for pituitary pars intermedia dysfunction (PPID)

Thesis submitted in accordance with the requirement of the University of Liverpool for the degree of Doctor in Philosophy

By

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I declare that the thesis has been composed by myself and that the work has not be submitted for any other degree or professional qualification. I confirm that the work submitted is my own, this includes study design, data analysis and interpretation, and preparation of the manuscript, except where otherwise indicated or where work which has formed part of jointly-authored publications has been included.

Rebecca Tatum

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Abstract

Older horses now make up a significant proportion of the equine population, meaning that geriatric medicine is increasingly important. Pituitary pars intermedia dysfunction (PPID) is a progressive disease prevalent in older horses, however, studies investigating diagnosis, treatment and prognosis are limited. The evidence base needs to be built on to inform management and treatment approaches and improve quality of life (QoL) of horses with PPID. The aims of this thesis were to i) identify and prioritise key research questions regarding PPID; ii) investigate management, health care and treatment strategies undertaken by owners iii) understand owner perceptions of treatment and QoL. A four-phase mixed methods approach was applied. Phase one used patient and public involvement (PPI), to prioritise the top 10 unanswered research questions for PPID. The questions focused on disease progression, diagnostic accuracy, treatment efficacy and additional management/treatment strategies. Literature on PPID diagnosis and treatment was subsequently evaluated in two systematic reviews, which highlighted the lack of high-quality evidence regarding current protocols. Phase two comprised a cross-sectional questionnaire to evaluate management and treatment strategies undertaken by 374 participating owners of PPID cases. Most horses were treated with pergolide (86.9%), while 17.4% received an alternative/complementary treatment. Pergolide had a higher efficacy rating compared to alternative/complementary treatments ($p<0.001$). PPID cases were perceived to have a very good QoL (median rating 9/10). Pergolide treatment was associated with an improvement in QoL rating since diagnosis ($p=0.002$). Phase three built on the cross-sectional data in a prospective cohort, using follow-up telephone questionnaires to describe changes over time. A subset of 72 horses were enrolled, contributing a total of 53.4 horse-years at risk (HYAR), with a median duration of follow-up of 330.7 days. For horses receiving pergolide (n=55), the dose remained the same throughout the follow-up period in 78.2%. QoL ratings remained unchanged in 43.5% of horses but decreased in 21.0%. Overall mortality rate was 20.6 deaths per 100 horse-years at risk (HYAR), while estimated incidence of laminitis was 15.7 new episodes per 100 HYAR and that of infection was 24.9 events per 100 HYAR. Finally, phase four used qualitative semi-structured interviews with ten purposively selected owners to understand key topics surrounding care and QoL for PPID cases. Six overarching themes were identified; disease tangibility, balancing management and treatment complexities, owners being experts in their own horse, having a horse centered approach, the vet-owner relationship and how health and happiness go 'hand in hand'. The themes demonstrated how owner decisions were influenced by the impact of PPID on the horse's daily life and the visible changes observed. This perception was then framed by the owners' understanding of the disease, their in-depth knowledge of what is normal for their horse and the nature of the vet-owner relationship. This thesis introduces the concept of PPI methodology in to equine veterinary research and provides novel information on the management, treatment and prognosis of PPID. It also highlights the lack of high-quality evidence on which clinical decisions are made. Pergolide treatment was associated with an increase in QoL but did not appear to influence morbidities or mortality. Owner experiences and perceptions play an important part in the treatment and management decisions made. The findings presented will help to develop effective management and treatment strategies to improve the QoL of horses with PPID. Owner perspectives and behaviours will also inform effective ways of communicating with owners about how to provide the best possible care.

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Preface

The aims of this PhD thesis are three-fold:

i) To identify and prioritise key research questions regarding the diagnosis, treatment and prognosis of pituitary pars intermedia dysfunction (PPID)

ii) To explore management and treatment strategies undertaken by owners of horses with PPID

iii) To gain an understanding of owner perspectives on treatment and quality of life (QoL) of horses with PPID.

The work presented here represents a mixed-methods approach to studying PPID. The chapters report findings from four studies. The initial study used patient and public involvement (PPI) methodology to identify and prioritise the key research questions about PPID that veterinary surgeons and horse owners want answering. Following this, evidence regarding current diagnosis and treatment protocols for PPID was assessed in two systematic reviews. Subsequent studies were developed to answer some of the important research questions prioritised in the initial PPI study. First, a cross-sectional study was designed to describe the management and treatment practices undertaken by owners and to investigate associations with quality of life (QoL). A subset of this population was then followed in a prospective cohort study to describe changes in management, treatment and QoL observed over time. Finally, to build on this, a qualitative approach was taken to provide an in-depth understanding of the role of horse owners in the management and treatment of PPID and the beliefs behind their decisions.

Chapter 1

Introduction and literature review

Introduction and literature review

The relationship between human and horse has changed in recent years. This shift is thought to be because the purpose of the horse has changed, and many are valued for their companionship (Hintz, 1995) through the development of feelings of mutual respect, affection and awareness of horses' individual needs (Dashper, 2017). Owners frequently develop a strong bond with their aged horse (McGowan and Ireland, 2016) and want to maintain their good health through the latter years of their life (Brosnahan and Paradis, 2003a). Surveys have demonstrated the willingness of owners to manage horses into their old age with 26-43% of aged horses kept as companions or retired while 62% are kept for leisure (Hintz, 1995; Ireland et al., 2011a; McGowan et al., 2010a). This means that, similar to the human population, horses are living longer. The median life expectancy of horses is estimated to be 22.7 years of age (Welsh et al., 2016) with an aged horse having been defined as ≥15 years (Mellor et al., 2001). This demographic now makes up a considerable proportion (25%-34%) of the equine population in western society (Mellor et al., 1999; McGowan et al., 2010a; Ireland et al., 2011a). However, increased longevity brings its own health implications (Cossio et al., 2012), such as the increased risk of disease.

Pituitary pars intermedia dysfunction

Pituitary pars intermedia dysfunction (PPID) is an age-associated progressive neurodegenerative disorder that results in a wide variety of clinical signs including hypertrichosis, muscle wastage and lethargy (McGowan et al., 2013a; Ireland and McGowan, 2018). It has also been associated with hyperinsulinemia and subsequently an increased risk of endocrinopathic laminitis (de Laat et al., 2019b). PPID has been diagnosed with increasing frequency in recent years (Rohrbach et al., 2012) and has an estimated prevalence of 21% among aged horses (McGowan et al., 2013a). It has also been reported to significantly affect risk of mortality (Welsh et al., 2016), making it important in geriatric medicine and equine practice.

A presumptive diagnosis of PPID has previously been based on the presence of specific advanced clinical signs such as generalised hypertrichosis (Frank et al., 2006a) however, in the earlier stages of disease, when clinical signs are more variable or less apparent, laboratory confirmation of diagnosis is required (Couëtil et al., 1996; Miller et al., 2008; McFarlane, 2011). Diagnosis and appropriate treatment at an early stage may be beneficial to help slow the progression of the disease (Roarty, 1990; McFarlane, 2007). The

measurement of basal adrenocorticotrophic hormone (ACTH) is currently recommended due to its ease and adequate accuracy when used with seasonal reference intervals (McGowan et al., 2013b). The thyrotropin-releasing hormone (TRH) stimulation test can also be used where a more sensitive test is required (Beech et al., 2007), however TRH is not currently licensed for use in horses.

Once diagnosed, the current licensed treatment is pergolide, an ergoline-based dopamine receptor agonist (Anon, 2011a). Medical treatment is often used in conjunction with various management changes to address the clinical signs present and overall health (Schott, 2002). The progressive nature of PPID means it requires long-term management often for a significant proportion of the horse's life; life expectancy post-diagnosis has been reported to be nearly 10 years (Welsh et al., 2016; Horn et al., 2019). The vast majority of management throughout this time is undertaken by the owner in the horse's home environment. This means that the part owners play in the in the management and treatment of the disease is vitally important and could have a significant impact on quality of life (QoL).

Pathophysiology

The exact pathophysiology of PPID is still unknown, however evidence suggests that oxidative stress contributes to neurodegeneration of the inhibitory dopaminergic hypothalamic neurons (McFarlane and Cribb 2005). Histological examination of the pituitary gland has provided evidence of increased oxidative stress markers in PPID cases compared to age-matched controls (McFarlane et al., 2005a). This leads to a loss of dopaminergic inhibitory output to the melanotrope cells within the pars intermedia of the pituitary gland. In turn, this results in increased plasma concentrations of multiple pro-opiomelanocortin (POMC) peptide derivatives including α-melanocyte stimulating hormone, corticotropin-like intermediate lobe peptide, β-endorphin and ACTH (Heinrichs et al., 1990; Dybdal et al., 1994; McFarlane, 2007; McFarlane, 2011; Durham, 2016a). The precise consequences of this are not fully understood (Durham, 2016a), however there a number of clinical signs associated with the disease which are described in further detail below.

Epidemiology

Risk factors

Increasing age is a significant risk factor for PPID, with one study reporting an odds ratio of 1.18 (95% CI 1.11–1.25; $p<0.001$) for every year increase in age among horses aged ≥15

years (McGowan et al., 2013a). Several other studies have also reported a significant association between PPID and increasing age. Horses with clinical signs of PPID were reported to be significantly older than those not showing clinical signs (Ireland et al., 2013). Similarly, owners reported PPID more frequently in horses aged ≥20 years compared to younger horses (Brosnahan and Paradis, 2003a) and the prevalence of PPID increased with increasing horse age in a referral hospital population (Brosnahan and Paradis, 2003b). Breed has been investigated as a risk factor for PPID in one study which compared diagnosis based on basal ACTH test results, and no significant associations were found (McGowan et al., 2013a). However, ponies were more likely to exhibit clinical signs than horses (McGowan et al., 2013a). The same study also investigated sex as a risk factor and found no significant difference in odds ratios between males and females.

Clinical signs

The foremost clinical signs associated with PPID are hypertrichosis, and other hair coat abnormalities (often incorrectly referred to collectively as hirsutism), weight loss, muscle wastage and signs of lethargy/depression-like state, while laminitis is also often reported as a comorbidity of PPID (McFarlane, 2011; McGowan et al., 2013a: Ireland and McGowan, 2018). However, the disease may manifest in different ways in individual cases (Hillyer et al., 1992; Schott, 2002). Clinical signs in the early stages of the condition can be subtle which then progress over time (Love, 1993).

Laminitis

Laminitis is a painful and debilitating disease (Bailey et al., 2006; Collins et al., 2010), which can have long term implications on the structures within the feet and subsequently athletic performance (Hood, 1999b; Hunt & Wharton, 2010). Endocrinopathic laminitis is associated with insulin dysregulation (Asplin et al., 2007; de Laat et al., 2010a; Meier et al., 2018) and has now been recognised as the most common form of laminitis (Karikoski et al., 2011). Studies have shown a significant link between laminitis and insulin dysregulation, including insulin resistance (IR) and hyperinsulinaemia (Treiber et al., 2006a; Bailey et al., 2008; Carter et al., 2009a; de Laat et al., 2010a; de Laat et al., 2010b). Insulin is a major regulatory hormone in glucose metabolism, and IR alters insulin signalling causing a reduction in glucose metabolism and subsequently hyperinsulinaemia (Bailey et al., 2006; Walsh et al., 2009; de Laat et al., 2010a). Studies have shown normal horses develop laminitis when hyperinsulinaemia is experimentally induced (Asplin et al., 2007; de Laat et

al., 2010b). Insulin resistance and hyperinsulinemia have been observed in horses affected by PPID (van der Kolk et al., 1995; McGowan et al., 2004; Treiber et al., 2006a; Klinkhamer et al., 2011) and they are thought to be at an increased likelihood of developing laminitis (Donaldson et al., 2004; Carter et al., 2009a; Karikoski et al., 2011).

The reported prevalence of laminitis amongst PPID cases varies widely between studies; one early case series reported the prevalence to be as low as 24% (van der Kolk et al., 1993), while a cases series from a university hospital reported a prevalence of 82% (Hillyer et al. 1992). Similarly, 74% of horses presenting with PPID at an ambulatory practice were reported to have laminitis (Donaldson et al., 2002). A recent systematic review assessed the epidemiology of PPID and calculated the overall prevalence of laminitis to be 48.9% (Ireland and McGowan, 2018).

Hypertrichosis and other hair coat abnormalities

Hypertrichosis, a long curly coat which fails to shed, is a 'classic' sign of PPID (Schott, 2002) and is considered a specific indicator of the disease (Frank et al., 2006a; McGowan et al. 2013a). One study has provided evidence this is caused by a greater percentage of hair follicles in the anagen stage of growth (Innerå et al., 2013). During the earlier stages of disease hair changes are more subtle, with regional changes often restricted to the lower jaw, base of the neck and palmar or plantar aspects of the limbs (Schott, 2002; Innerå et al., 2013). This can progress over time to the long curly coat characteristic of the advanced stages of PPID. The prevalence of veterinary observed hypertrichosis and/or hair coat abnormalities was reported to be 33% in a cross-sectional study of 69 aged horses and ponies with PPID (McGowan et al., 2013a). In the same study, coat changes were observed by 40.6% of owners, suggesting it could possibly be over-diagnosed by owners or that they are better placed to recognise changes over the year compared to a single veterinary exam. Conversely, veterinary-reported prevalence of coat changes as high as 94-100% have been reported in a clinical trial (McGowan and Neiger, 2003) and several case series (Hillyer et al., 1992; van der Kolk et al., 1993; Couëtil et al., 1996; Spelta and Axon, 2012). This variation is likely due to differences in selection criteria, time of year the study was performed and types of animals included; for example, ponies are more likely to show hair coat abnormalities than horses (McGowan et al., 2010b; Ireland et al., 2012c). An overall prevalence of 69.9% for all hair coat abnormalities reported amongst PPID cases has recently been calculated based data from 14 published studies (Ireland and McGowan, 2018).

Muscle atrophy

Muscle atrophy associated with PPID is characterised by loss of epaxial and gluteal musculature (McFarlane, 2011) and has been shown to affect type II fibres more than type I fibres (Aleman et al., 2006; Narici and Maffulli, 2010). Muscle atrophy as a result of aging (sarcopenia) is observed in equids and humans without underlying disease. It can therefore be mistaken as normal sign of aging (Hintz, 1995; Narici and Maffulli, 2010) and may not be recognised until its later stages (Schott, 2002). Muscle atrophy occurs when protein degradation rates exceed protein synthesis (Schiaffino et al., 2013). One study has suggested greater gene expression of m-calpain involved in proteolysis may be one of the mechanisms which plays a role in the development of muscle atrophy in PPID (Aleman and Nieto, 2010). Muscle atrophy or epaxial muscle wastage are reported to have a prevalence of 18-50% in PPID cases (Pongratz et al., 2010; Spelta and Axon, 2012; McGowan et al., 2013a).

Abnormal fat distribution

Abnormal fat distribution typically comprises the development of regional adiposity including fat deposits above the eyes in the supraorbital fossa, along the crest of the neck and over the tail head. The process underlying tissue relocation to the upper parts of the body is not fully understood, however, there may be a relationship between the redistribution of fat and hyperinsulinemia seen in a proportion of PPID cases (Frank et al., 2006b; Carter et al., 2009b; McFarlane, 2011). Redistribution of body mass is a commonly reported clinical sign (Donaldson et al., 2004). Bulging supraorbital fat is reported in 6-50% of cases (Hillyer et al., 1992; van der Kolk et al., 1993; Couëtil et al., 1996; Donaldson et al., 2002; Perkins et al., 2002; McGowan and Neiger, 2003; Donaldson et al., 2004; Spelta and Axon, 2012; McGowan et al., 2013a).

Lethargy

Lethargy, or a depression-like state, is a common sign of illness in horses (Hausberger et al., 2016). It is characterised by prolonged times of immobility, withdrawn or "slumped" posture and a lack of response to environmental stimuli; however, there is no standardised measure (Burn et al., 2010; Fureix et al., 2012). Changes to circulating neurosteroids, similar to that seen in humans with Parkinson's disease, have been observed in horses with PPID and it has been hypothesised that this may contribute the lethargic state observed (Schott et al., 2020a). Owners reported lethargy/depression-like state in 14.5% of horses diagnosed with

PPID in a cross-sectional study (McGowan et al., 2013a). However, the prevalence reported during veterinary examination among other studies varies from 3-95% (Hillyer et al., 1992; Couëtil et al., 1996; Donaldson et al., 2002; Perkins et al., 2002; McGowan and Neiger, 2003; Donaldson et al., 2004; Pongratz et al., 2010). Overall prevalence has been estimated to be 41% (Ireland and McGowan, 2018).

Diagnosis of PPID

Previously, a presumptive diagnosis based on age and evidence of hypertrichosis, associated with the more advanced stages of disease, was considered adequate (Frank et al. 2006a; Schott, 2002). However, acknowledgement that PPID is a progressive disease with varying clinical signs (McGowan et al. 2013a; McGowan et al., 2013b) and the suggestion that early intervention might have a protective effect (Gille et al., 2002) meant there was a need to identify the disease in the early stages. Clinical diagnosis can be challenging in the early stages of disease when signs of hypertrichosis may not be evident or in cases with less specific clinical signs (Miller et al., 2008). Therefore, a number of different endocrinologic assay tests have been developed and used in the diagnosis of PPID, including basal and dynamic tests (Frank et al. 2006a; McGowan et al. 2013b). Current diagnostic tests for PPID that are considered to possess adequate accuracy and availability for clinical use comprise basal plasma ACTH concentration, the overnight dexamethasone suppression test (ODST) and the thyrotropin-releasing hormone (TRH) stimulation test (measuring ACTH) (Durham et al., 2014). However, there is a lack of a 'gold standard' laboratory test (McGowan et al., 2013b).

Basal plasma ACTH concentration

Production of ACTH in horses normally occurs in corticotrope cells in the pars distalis, which release the hormone in response to corticotropin, following cleavage from the peptide POMC (Wilson et al., 1982; García-Borrón et al., 2005; Getting, 2006; Yang, 2011). Horses with PPID have elevated levels of ACTH released into plasma from the pars intermedia melanotropes (Couëtil et al, 1996; Durham et al., 2014) which is thought to gradually increase as the disease progresses (McGowan et al., 2013b). The activity of the pars intermedia melanotropes is regulated by hypothalamic and systemic neurochemicals, including dopamine (Shioda et al., 1987; Saland, 2001; McFarlane et al., 2003; McFarlane et al., 2005b).

Plasma ACTH concentration compared to seasonally adjusted reference intervals (RIs) offers a simple test requiring only a single sample to be collected (Rendle et al., 2015a) and has been evaluated in several small studies with varying accuracy reported. However, these studies are limited by small sample sizes and there is a lack of standardisation to protocols, meaning different assays and definitions of disease were used, making comparison challenging. The accuracy of the basal ACTH test for diagnosing PPID is examined in detail in Chapter 3. However, ACTH concentrations can be affected by several in vivo and in vitro factors. Stress brought on by transportation has been shown to significantly increase ACTH levels in stallions (Fazio et al., 2008). A small study of five horses described that ACTH levels were significantly affected for up to 30 minutes after treadmill exercise (Nagata et al., 1999) and a similar effect has also been observed in humans (Buono, 1986). Incorrect sample handling and storage can also affect plasma ACTH levels. If samples are not promptly chilled or frozen ACTH levels have been shown to progressively decrease in horses with PPID (Perkins et al., 2002; Durham and Copas, 2011), and significantly decrease after 4-8 hours (Rendle et al., 2015b). It has also been reported that pain leads to an activation of the hypothalamic-pituitary-adrenal axis, resulting in the release of ACTH and cortisol from the pars distalis, in both livestock species (Molony and Kent, 1997) and horses (Beech et al., 2007). A recent small case control study examined 17 horses, aged ≤15 years of age and with no clinical signs of PPID, that were suffering from pain (Gehlen et al., 2020). Pain scores were established and blood samples were taken at initial examination and again when the horse was considered to be pain free. A correlation between higher pain score and increased ACTH level was reported however, this was not statistically significant, possibly due to the small sample size.

Seasonality is also an important consideration. An autumn increase in ACTH was first described by Donaldson et al, (2005), who reported significantly higher levels in September compared to January and May. This circannual rhythm was further investigated by Copas and Durham (2012) who demonstrated that the autumn rise in ACTH was magnified in horses with PPID compared to controls. The same study proposed seasonal RIs to allow testing year-round. However, borderline results in the 'grey zone' (commonly reported as between 20 and 40pg/ml) close to the autumn or non-autumn cut-off should be interpreted with caution alongside clinical history and retesting at a later date or using a different secondary test may be considered (McGowan et al., 2013b; Rendle et al., 2014; Rendle et al., 2015a).

Overnight dexamethasone suppression test (ODST)

The ODST test measures cortisol concentrations 18–20 hours following dexamethasone administration (Dybdal et al., 1994). In horses with PPID, significant amounts of ACTH are produced by the pars intermedia, which means that cortisol secretion is maintained after administration of exogenous glucocorticoids. This test was previously considered the gold standard for the diagnosis of PPID with 100% sensitivity and specificity reported (Dybdal et al., 1994). However, this was later brought into question by Frank et al, (2006) who reported a sensitivity of 65% and specificity 76% (Frank et al., 2006a) and another small study which reported a lack of repeatability (Miesner et al., 2003). Furthermore, the ODST test has other limitations and is no longer recommended for use (EEG, 2019); the test requires two samples to be taken on two consecutive days, prior to and then following dexamethasone administration, incurring additional costs. Moreover, there are concerns around the administration of corticosteroids in horses at an increased risk of laminitis (Potter et al., 2019).

Thyrotropin-releasing hormone (TRH) stimulation test

The TRH stimulation test was developed to try and improve the diagnosis of early or mild cases, which the basal ACTH may not be sensitive enough to identify. The presence of messenger ribonucleic acid (mRNA) for TRH Type 1 receptors has been reported in the pars intermedia melanotropes and pars distalis corticotropes in normal horses and horses with PPID (McFarlane et al., 2006). Administration of TRH has been shown to increase plasma concentrations of ACTH in horses with a significantly greater response in horse with PPID compared to normal horses (McFarlane et al., 2006; Beech et al., 2007; 2011a,b). Currently, two small studies have reported the TRH stimulation test to have a sensitivity of 95-100% (Beech et al., 2007, 2011b). As with the basal ACTH test, seasonal variation affects test results and seasonal reference intervals have been developed (Diez de Castro et al., 2014). The TRH stimulation test is generally considered a second-tier test which is used to confirm borderline basal ACTH test results or when other basal diagnostic test results are not in agreement with observed clinical signs. TRH is not currently licensed for use in horses and is therefore only permitted for use via the prescribing cascade (Anon, 2013).

Treatment and management of PPID

Pergolide, a long-acting dopaminergic agonist which is thought to reduce the secretion of precursors of ACTH, is currently the only PPID treatment licensed for use in horses (Muñoz

et al., 1996; Anon, 2011a). Other treatments such as cyproheptadine, a serotonin antagonist, and bromocriptine, another dopaminergic agonist, have also been considered as treatment options. However, less supportive evidence has been published for these medications (Roarty, 1990; Beech, 1994; Donaldson et al., 2002; Perkins et al., 2002) and there are no licenced products available.

Pergolide has been described as effective at decreasing ACTH concentrations and improving clinical signs of disease in several studies. In particular, a large field study of 113 cases described improvement in clinical signs and/or endocrine test results in 76% of horses following treatment with 2-4µg/kg pergolide for 180 days (Anon, 2011a). The efficacy of pergolide for the treatment of PPID is examined in detail in Chapter 4. In brief, studies are predominantly limited to hospital-based populations and no studies have formally assessed the impact of management changes. Regardless of the level of evidence available, the treatment approaches taken for any disease are predominantly dictated by the owner (Christley and Perkins, 2010) and the treatment decisions made can be influenced by a multitude of factors such as their own experiences and perceptions (Scantlebury et al., 2014).

Due to the disease's progressive nature and varying clinical signs (Heinrichs et al., 1990; Miller et al., 2008; McGowan et al., 2013a) it is likely that the effective dose of pergolide varies between individuals. Current recommendation is a starting dose of 0.002mg/kg (Peters et al., 1995; Schott et al., 2001; Donaldson et al., 2002; Durham et al., 2014). Side effects such as decreased appetite, weight loss, depression-like state, sweating and dyspnoea have been reported when horses are started on a higher dose (Anon, 2011a; Muñoz et al., 1996; Schott et al., 2001). Time until a detectable response to treatment is expected to be observed and when dosage should be increased has not been well documented. Current recommendations are for treatment to be reviewed monthly until the horse is considered to be stabilised and then review 2 – 4 times a year (Durham et al., 2014). In one study, 22% of owners discontinued treatment due to lack of response (Rohrbach et al., 2012) therefore effective monitoring is likely to be a necessary part of treatment. If pergolide is discontinued, ACTH levels increase considerably after 2-10 days (McFarlane et al., 2017) suggesting life-long daily dosing is required.

Treatment of PPID also includes consideration of general health care and management changes as this notably impacts health and quality of life (QoL). This may include regular

dental care and in the latter stages, clipping to remove excess coat (Schott, 2002). Changes to diet and daily routine are also likely to be required to reduce the risk of laminitis in horses with concurrent insulin dysregulation and to maintain a healthy body condition. Management practices carried out by owners have been reported to change as horses age, with exercise intensity reported to decrease in the majority of cases (60.8%), an increase in the use of veteran and hay replacer feeds and less preventive health care administered (Ireland et al., 2011a,b). It is therefore important to understand the treatment and management practices undertaken by owners of horses with PPID and the level of compliance with veterinary recommendations, as these factors are likely to impact treatment efficacy.

There is little evidence available regarding long-term prognosis of horses with PPID. However, it seems horses can live for long periods of time following diagnosis. One study followed horses for 5.5 years and reported 40% of horses survived to last follow-up (Schott et al., 2014), while another small study reported 50% of horses survived 4.5 years after diagnosis (Rohrbach et al., 2012). However, a recent study reported that a diagnosis of PPID was associated with an increased risk of mortality, although median life expectancy after diagnosis was 9.8 years (Welsh et al., 2016). A recent retrospective study had a maximum follow-up period of 7 years; however, the median follow-up time was only 11 months (Horn et al., 2019). This study was unique in also following non-treated horses and reported improvement (as per attending clinician) in 64.3% of horses treated with pergolide and 6.7% of non-treated horses. Both pergolide treatment and clinical improvement at re-examination were positively associated with survival ($p=0.01$) (Horn et al., 2019). There remains a lack of long-term or prospective cohort studies.

Quality of life

As a chronic disease that can affect horses for a large proportion of their life, it is important that both veterinary surgeons and owners are able to ensure horses with PPID enjoy a good QoL. The World Health Organisation defines health as "A state of complete physical, mental, and social well-being not merely the absence of disease" and QoL is a measure that encompasses 'wellbeing' and not just changes in the frequency or severity of disease (WHO, 1997), however a consistent definition is lacking (Rapley, 2011). Health status has been used a proxy for QoL, however health problems do not necessarily directly relate to lower QoL ratings (Carr and Higginson, 2001). In veterinary medicine, the term QoL is often used synonymously with the term "welfare", however the consensus is that QoL goes beyond

welfare to encompass psychological health and general enjoyment of life (Broom, 2007). It has been proposed that QoL encompasses three main elements:

1) Positive and negative feelings, ranging from severe pain to playfulness

2) Physical fitness and health, encompassing elements such as how a disease affects their day to day life and physical ability to cope with its environment

3) Naturalness, the ability to carry out natural behaviours and experience elements of natural environments (Fraser et al., 1997)

The emphasis placed on the different aspects of QoL varies between social groups; for instance farmers put an emphasis on health, while welfare scientists think feelings are most important (Kling-Eveillard et al., 2007; Prickett et al., 2010). There has been little investigation into how veterinary surgeons or horse owners perceive QoL in horses. One study asked owners of geriatric horses to rate QoL and reported that the vast majority of owners perceived their horse to have a good QoL (Ireland et al., 2011c). It has been acknowledged that owners play an important role in assessing the day to day QoL of their horse (Parker and Yeates, 2012) and have more experience of their horse as an individual (McMillan, 2000, 2003). It is therefore important to understand more fully how owners perceive QoL in their horses, both when healthy and with disease or injury.

In human medicine, QoL assessment has been long established and used to successfully measure the effect of chronic conditions and the implications of aging as well as the effect of various interventions (Schlenk et al., 1997; Skevington et al., 2001; Hyde et al., 2003, 2015; Megari, 2013; Van Es et al., 2013). Similar forms of QoL measures have subsequently been established and validated for small animal veterinary medicine, becoming an important part of veterinary assessments. Questionnaires and QoL scales have been developed to measure the effect of diseases such as epilepsy in dogs (Wessmann et al., 2014), chronic pain in dogs (Wiseman-Orr et al., 2004), heart disease in cats (Reynolds et al., 2010) and cancer in both cats and dogs (Lynch et al., 2011). QoL has also been used to assess the effects of treatments such as chemotherapy and palliative care (Mellanby et al., 2003; Tzannes et al., 2008). Owner evaluation of QoL has also been shown to be an important and effective way of assessing management (Lavan, 2013; Tatlock et al., 2017). Various types of pain assessment tools have been described for different types of pain in horses including composite pain scores for colic (van Loon et al., 2015; Van Dierendonck and van Loon,

2016) and facial expression pain scores for laminitis (Dalla Costa et al., 2016) and orthopaedic pain (Dyson et al., 2017; Mullard et al., 2017; van Loon et al., 2019). However, currently there is no standardised means of measuring QoL in horses. The ability to properly assess individual QoL can help identify areas for improvement and have clear benefits (Pitkänen et al., 2009) and is clearly an area in which equine medicine needs to advance.

Sociological research in equine veterinary medicine

Although some advances in the diagnosis and treatment of PPID have been made in recent years, ultimately it is the owners who mediate the diagnosis, treatment and care of their horses (Christley and Perkins, 2010). It is therefore important that veterinary surgeons understand the lay beliefs behind the decisions that owners make and the practices they undertake. Qualitative interviews can be utilised to shed light on what influences owner decision making around approaches to the treatment and management of PPID (DiCicco-Bloom and Crabtree, 2006). Such methods are important in investigating PPID from a novel perspective. In human medicine, sociological research is well established and forms an integral part of a patient centred approach. Although there has been an increase in qualitative sociological studies within veterinary medicine, they remain relatively scarce in equine veterinary medicine. This suggests further understanding of qualitative techniques and how these can be practically applied to equine medicine is needed.

Owner perspectives of health in the older equid

How owners rate the importance of their horse's health issues as they age can provide some insight into how they perceive various diseases. McGowan et al, (2010b) reported what owners perceived as the most important health issues among older horses, with loss of condition, arthritis/lameness and teeth/dental care of particular concern. QoL and management factors around caring for their horse were also frequently reported welfare concerns. Knowledge of owner health priorities among older horses can be useful in focusing messaging or increasing awareness of diseases; however, it does not provide any understanding or context around why the diseases were ranked as important.

Diagnosis of a disease in the first place relies on owners recognising clinical signs in their animals. Two studies have investigated owner perception of clinical signs in geriatric horses. Ireland et al, (2012a) found that owners under-reported many clinical signs of disease and there was poor agreement between what owners reported and subsequent veterinary

assessment. Another study similarly reported that owners readily described clinical signs but reported the actual disease much less frequently (McGowan et al., 2010b). This suggests that owners under-recognise clinical signs in their horses and when they do recognise them, they do not necessarily attribute them to a disease. This could lead to significant delays in seeking veterinary attention.

A further study investigated factors affecting treatment decisions made by owners of older horses, and Ireland et al, (2011d) reported that post-procedure prognosis, the seriousness of the disease, pain associated with procedures and veterinary advice were the biggest influencers (Ireland et al., 2011d). Although this provides some useful information regarding owner decision making and considerations veterinary surgeons should make when discussing options with owners, it does not provide the level of context or depth of information required to fully understand owner perspectives or what influences them.

Lay beliefs and perceptions

A qualitative, interview-based study investigated how horse owners perceived the health and performance of Pony Club horses (Buckley et al., 2004). The high value that owners placed on their horse looking visibly healthy with "bright eyes, shiny coat and good body condition" was highlighted. This study also gave some insight into how owners acquired information regarding their horse health. The advice of 'knowledgeable friends' was considered an important point of contact, compared to veterinary surgeons who were only consulted for what were perceived as serious conditions (Buckley et al., 2004). This demonstrates the important influence of the horse-owning community to which owners belong and highlights where education and advice need to be communicated. Another study utilised a unique Delphi methodology to score vignettes of scenarios based on the themes 'essentials, physical insult and psychological injury'. Rounds of Delphi surveys with key opinion-formers were used to identify important welfare issues among horses in Ireland and how welfare standards could be raised (Collins et al., 2010). The study used the novel approach of scoring vignettes and identified broad areas of concern such as unregulated gatherings. However, more importantly, it highlighted the need to understand the motivators for and barriers against improving welfare standards among owners if interventions are to be successful. Another focus group based study investigated the perceptions of stakeholders in the racing industry (Butler et al., 2019). This study differed slightly as it included both professional and lay participants such as veterinary surgeons, trainers, staff and owners. Thematic analysis highlighted how stakeholders divided what was needed for a horse to 'live

its best life' and what was considered a 'minimum welfare standard'. The importance of available veterinary care, correct feed and management and allowing the horse to exhibit natural behaviours were all identified alongside training and exercise.

Owner management and treatment practices

Owner management strategies and beliefs surrounding these can vary widely. This was highlighted by a telephone-questionnaire based study investigating management of horses with stereotypical behaviours on different types of yards (McBride and Long, 2001). The study found significant differences between the type of establishment and how owners perceived and managed horses with stereotypical behaviours, including use of preventative measures, effect on performance and the underlying cause of the behaviour. Differences in management between amateur and professional horse owners such as how horses are housed and fed have been described (Harris, 1999). This paper also described owners would forgo things, such a holiday, to ensure their horse got what they needed. This again highlights the need to further understand the motivators and decisions of horse owners when it comes to the management and treatment of their horses. An Australian study analysed qualitative questionnaire responses to examine the barriers to owners vaccinating against Hendra virus (Manyweathers et al., 2017). The low perceived Hendra virus risk, vaccine safety and cost of vaccination were reported to be important barriers. Interestingly most owners would not consider vaccination even when advised by their veterinary surgeon. This shows the influence of owner perceptions when it comes to veterinary treatment and the importance of a trusting owner-vet relationship when it comes to animal welfare.

The management and treatment of horses has been reported to change as they age, with a reduction in preventive health care and routine veterinary treatment described (Ireland et al., 2011b). A recent cross-sectional study used Likert-scale questions to assess owner confidence in managing and recognising disease in older equids (Bushell and Murray, 2016). Although owners had a high level of confidence with regards to management regimes, confidence in disease recognition was lacking. Reduction in veterinary interactions and difficulty recognising disorders is likely to have a detrimental effect on welfare and QoL.

Evidence-based medicine

The term 'evidence-based medicine' (EBM) was first introduced to scientific literature in the British Medical Journal by Guyatt et al., in the early 1990's and is defined as "the

conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients" (Guyatt et al., 1992). Since then the principles have been rapidly adopted, having a significant effect on implementing and teaching medicine (Guyatt et al., 1992; Bernstein, 2004; Mellis, 2015), integrating clinical expertise with evidence from systematic research (Sackett and Rosenberg, 1995; Sackett, 1997; Heneghan and Badenoch, 2006).

EMB is underpinned by a process of five steps (Heneghan and Badenoch, 2006):

1. Ask an answerable clinical question (by defining the patient/problem, intervention, comparison/control and outcomes, known as the PICO format).

2. Acquire the best evidence.

3. Appraise the evidence critically.

4. Implement the evidence into practice where appropriate.

5. Access the impact.

The EBM process can be directly applied to equine veterinary medicine. However, the concept of evidence-based veterinary medicine (EBVM) is still fairly new (Dean, 2014) and has been met with a number of challenges. These include concerns about the scarcity of high-quality evidence, a lack of time to search for the evidence, lack of training/skills to appraise it and that clinical experience and expertise may be overlooked (More, 2012; Vandeweerd et al., 2012; Dean, 2014; Turner and Royle, 2015).

The first step in improving the evidence base is to identify the clinical questions veterinary surgeons and horse owners need answering. As discussed previously, PPID has become increasingly important in equine medicine, however the availability of high-quality evidence regarding its diagnosis, treatment and prognosis is limited. EBM assesses types of evidence according to the probability of bias, with systematic reviews and randomised clinical trials considered the most robust studies, while case series or expert opinions are the lowest level (Phillips et al., 2009). Until very recently systematic reviews were scarce in veterinary medicine however their importance is beginning to be recognised. One has recently examined the epidemiology of PPID (Ireland and McGowan, 2018) and systematic reviews

conducted as part of the research in this thesis evaluated the diagnosis (Chapter 3) and treatment (Chapter 4) of PPID. Prior to this, the evidence pertaining to PPID was predominantly based on less robust case reports/series and case control studies from predominantly small hospital-based populations, which are not necessarily generalisable to the general population. This thesis builds on the evidence base to better inform veterinary surgeons and ensure the QoL of horses with PPID.

Patient and public involvement

Patient and public involvement (PPI) has been defined as "Healthcare professionals working together with patients and the public to improve the health communities they serve" (Cartwright and Crowe, 2011). It encompasses a broad range of methods to engage heath care end users in the process of determining what research is done, how it is conducted and how the findings are interpreted and used (Adamson et al., 2005). People living with health problems and those who use health services can make useful contributions to widen perspectives, bridging the gap between patients and researchers. This was first recognised in the 1990's (Chalmers, 1995; Oliver, 1995; Entwistle et al., 1998) and the scope for patient involvement to aid identification of important questions for research was also recognised (Chalmers, 1995; Oliver, 1995; Entwistle et al., 1998). It has been acknowledged that researchers do not always investigate areas that are important to the end users. PPI encourages research agendas to be broadened and to encompass the 'voice of the patients', with the aim of improving the delivery of health care and ensuring the evidence base is representative of the consumer (Tallon et al., 2000; Oliver et al., 2001; Chalmers et al., 2012). This has led to initiatives such as INVOLVE designed to support active public involvement in the National Health Service (NHS) by carrying out research 'with' the public rather than 'about' them (INVOLVE, 2017). In theory, PPI methodology could easily be adapted into an equine veterinary setting to ensure that future research is clinically relevant to the end users such as veterinary surgeons and owners.

The James Lind Alliance framework

The James Lind Alliance (JLA) framework is used in human medical research to provide a platform for an independent and integrated approach to PPI, involving patients, carers and clinicians in setting research priorities (Elwyn et al., 2010; Lophatananon et al., 2011; Cowan, 2013; JLA, 2016). The freely available framework provides guidance on establishing and managing Priority Setting Partnerships (PSPs), with the aim of raising the awareness

and funding of research questions which have a direct relevance and benefit to patients and the clinicians who treat them (JLA, 2016).

The JLA methodology uses a mixture of quantitative and qualitative approaches to identify and prioritise gaps in the current available evidence known as 'uncertainties'. Uncertainties occur when questions about diagnosis, treatment and prognosis cannot be answered by up to date information based on research evidence (JLA, 2016). The methodology includes surveys to identify uncertainties and an adapted Delphi method to undertake prioritisation (Pill, 1971; Gordon, 1994; Lophatananon et al., 2011). PSPs are structured using a nominal group technique to ensure equal contributions from patients and clinicians to efficiently prioritise the uncertainties identified (Jones and Hunter, 1995), resulting in a prioritised 'top 10' of research questions that need answering. This technique is underpinned by principles of a clear audit trail, transparency and a commitment to using and contributing to the evidence base (JLA, 2016). The first PSP in 2007 identified the top 10 research questions for asthma (Elwyn et al., 2010). Since then, the technique has been used in many further publications making it a tried and tested method (JLA, 2016). A PSP was previously utilised to identify and prioritise treatment uncertainties for chronic kidney disease in cats (Dean, 2014). Otherwise, the JLA framework has rarely been used in veterinary medicine as a whole, and to date has not been used in equine veterinary medicine.

The JLA framework has been used to identify priorities for Parkinson's disease, which is thought to share pathologic similarities to PPID. Parkinson's disease is also an age-associated neurodegenerative disease. It occurs sporadically in the geriatric human population. Neurodegeneration of the nigrostrial dopaminergic neurons results in a loss of inhibitory neurotransmitter dopamine in the striatum. This leads to the motor dysfunction observed in Parkinson's disease (McFarlane, 2007). The priorities identified included:

- What is the best method of monitoring a person with Parkinson's response to treatments?
- What drug treatments are best for the different stages of Parkinson's?
- Can we develop accurate tests/techniques to determine medication timing and amount (dose)? (JLA 2016 online archive)

This example demonstrates several parallels to treatment and monitoring considerations for horses with PPID, thereby indicating that the JLA method could readily be adapted from human to equine medicine in order to prioritise research questions pertaining to PPID.

Overall, current literature regarding the diagnosis, treatment and prognosis of PPID is limited in terms of the populations studied and outcome observed. The evidence base needs to be built on, and PPI methodology can be adapted to help prioritise and direct areas of research to fill evidence gaps and ensure future research is applicable to end users. Due to the long-term management horses with PPID require, it is important to understand the management and treatment practices made by the owners that care for them to help improve QoL. Sociological based research can contribute to a more evidence-based approach to management and treatment, by understanding owner perceptions of QoL and decision making around treatment.

Ojectives of the thesis

1. To adapt PPI methodology in order to identify and prioritise key research questions regarding the diagnosis, treatment and prognosis of pituitary pars intermedia dysfunction (PPID)
2. To systematically collate and evaluate the evidence regarding diagnostic and treatment protocols for PPID
3. To identify and evaluate management and treatment strategies undertaken by owners of horses with PPID and how these change over time
4. To gain an understanding of owner perceptions of and decision making around treatment and QoL in horses with PPID

In order to achieve these objectives, quantitative and qualitative studies were undertaken in a population of horses diagnosed with PPID:

Chapter Two describes the adaptation of the JLA framework to identify research priorities for the diagnosis, treatment and prognosis of PPID.

Chapter Three systematically collates and assess the evidence regarding measurement of basal ACTH for the diagnosis of PPID.

Chapter Four systematically collates and assess the evidence regarding the use of pergolide for the treatment of PPID.

Chapter Five describes the management and treatment practices undertaken by owners of horses diagnosed with PPID as well as perceptions of QoL, utilising a cross-sectional online questionnaire.

Chapter Six prospectively follows a subset of horses from the cross-sectional study over a 12 month period using follow-up telephone questionnaires. Rates of mortality and disease incidence are described as well as changes in owner management and treatment strategies and perceptions of QoL.

Chapter Seven uses semi-structure interviews to gain an in-depth understanding of owner decision making around the treatment of PPID and perceptions of QoL.

Chapter Eight summarises the information gained from these studies, discusses practical applications for these findings and highlights areas that merit further research.

Chapter 2

Equine pituitary pars intermedia dysfunction: Identifying research priorities for diagnosis, treatment and prognosis through a priority setting partnership

Equine pituitary pars intermedia dysfunction: Identifying research priorities for diagnosis, treatment and prognosis through a priority setting partnership

The following chapter comprises the James Lind alliance Priority Setting Partnership, and is presented as it was published in PLOS ONE.

Reference: Tatum RC, McGowan CM, Dean RS, Ireland JL (2021) Equine pituitary pars intermedia dysfunction: Identifying research priorities for diagnosis, treatment and prognosis through a priority setting partnership. PLOS ONE 16(1)

Summary

Pituitary pars intermedia dysfunction (PPID) is the most prevalent endocrine disorder of older equids. To date, key research areas likely to have the greatest impact on equine health have not been identified. In human medicine, public and patient involvement is widely used to inform research agendas. This study aimed to engage with veterinary surgeons and horse owners to identify evidence gaps ('uncertainties') and prioritise these into a list of the 10 most important PPID research questions. The James Lind Alliance (JLA) Priority Setting Partnership (PSP) Framework was adapted. Questions about the diagnosis, treatment and prognosis of PPID were gathered via an online survey targeting veterinary surgeons and horse owners with experience of PPID. Thematic analysis was used to form a longlist of collated indicative research questions (CIRQs), defined by the JLA as true 'evidence uncertainties' when not answered by a published, clinically relevant, up-to-date systematic review. In an interim prioritisation survey, questions were ranked by weighted scores creating a shortlist of 25 that were taken forward to the PSP workshop, where participants reached a consensus on the top 10. Useable responses containing ≥1 question were received from 524 respondents (92.6% owners, n=485; 7.4% veterinary surgeons, n=39). After screening for relevance, 1,260 individual questions were included in thematic analysis, resulting in 47 CIRQs. Interim prioritisation votes for the CIRQs were received from 360 respondents. The top 10 questions prioritised at the PSP workshop focused on long-term prognosis, diagnostic accuracy, efficacy of pergolide treatment, alternative treatment/management strategies and potential treatment options for poor responders to pergolide. The quantity of questions generated indicates an extensive number of uncertainties regarding the diagnosis, treatment and prognosis of PPID. The top 10 research questions will help to inform key areas for evidence synthesis and knowledge translation,

and to direct future research into areas most important to end users involved in caring for and treating animals with PPID.

Introduction

Pituitary pars intermedia dysfunction (PPID) is a common age-associated equine neurodegenerative disorder (McFarlane and Cribb, 2005; McGowan et al., 2013a). While the exact pathophysiology of the disease remains poorly understood (McFarlane, 2007; Miller et al., 2008), oxidative stress is thought to contribute towards progressive neurodegeneration of the inhibitory dopaminergic hypothalamic neurons (McFarlane and Cribb, 2005; McFarlane et al., 2005c). This leads to a loss of dopaminergic inhibition of the pars intermedia lobe of the pituitary gland and over production of pituitary-derived hormones. As a result, increased plasma concentrations of pro-opiomelanocortin (POMC) peptide and its derivatives, including α-melanocyte stimulating hormone, corticotropin-like intermediate lobe peptide, β-endorphin and adrenocorticotrophic hormone (ACTH), are observed (Heinrichs et al., 1990; McFarlane et al., 2005c; McFarlane, 2007). This proliferation of hormones is associated with a variety of clinical signs and comorbidities including hypertrichosis, laminitis, epaxial muscle wastage or muscle atrophy and lethargy (Schott, 2002; McFarlane, 2011; McGowan et al., 2013a; Ireland and McGowan, 2018). Current published evidence about diagnosing and managing PPID is limited, both in terms of the study populations included and the outcomes measured. Various different endocrinologic assay tests have been developed for the diagnosis of PPID. However, a 'gold standard' laboratory test is currently lacking (Frank et al., 2006a; McGowan et al., 2013b). Once diagnosed, the dopamine agonist pergolide is currently the only licensed treatment for PPID (Muñoz et al., 1996; Anon, 2011a). However, evidence regarding its efficacy is largely based on a single uncontrolled trial (Anon, 2011a) and numerous descriptive reports (Peters et al., 1995; Muñoz et al., 1996; Sgorbini et al., 2004; Aleman et al., 2006; Rohrbach et al., 2012; Spelta and Axon, 2012). Therefore, a more robust evidence base is required to inform veterinary surgeons and horse owners regarding optimal methods for diagnosis and medical treatment of PPID.

Ensuring that research is relevant and applicable to those who can improve patient care is essential. However, researchers do not always investigate areas or answer questions important to the end users (Tallon et al., 2000; Oliver et al., 2001). Inclusive research methods in medicine have been developed to bridge the gap between patients, clinicians and researchers, encouraging widening of perspectives to identify gaps in the evidence and prioritise research agendas (Chalmers, 1995; Oliver, 1995; Entwistle et al., 1998). One of

these methods is the framework developed by the James Lind Alliance (JLA) Priority Setting Partnership (PSP). The JLA has been established as a platform for an independent integrated approach to setting research agendas (Petit-Zeman et al., 2010; JLA, 2017). It brings together patients, carers and clinicians on a "level playing field" to identify and prioritise unanswered research questions, known as 'uncertainties' (Elwyn et al., 2010; Lophatananon et al., 2011; Cowan, 2013; JLA, 2016, 2017). Despite successful public and patient involvement (PPI) being used in priority setting for human medical research for over a decade (Entwistle et al., 1998; Adamson et al., 2005), it has only recently been adapted for use in a veterinary setting. An adaptation of the JLA framework has previously been used to set priorities for research into the treatment of chronic kidney disease in cats (Dean, 2014). However, to date such priority setting has not been applied to equine research.

Veterinary surgeons and horse owners with experience of PPID are best placed to identify questions about the disease most in need of answering. Therefore, the aim of this study was to engage these end users in order to identify their top 10 research priorities for the diagnosis, treatment and prognosis of PPID.

Materials and methods

Adaptation of the James Lind Alliance (JLA) Framework

This study did not include animal participants, therefore international, national, or institutional guidelines for humane animal treatment are not applicable. This project received institutional ethical approval from the University of Liverpool and Animal Health Trust. Following institutional ethical approval, the six steps of the JLA framework (Petit-Zeman et al., 2010) were adapted to identify research priorities for PPID:

- Identification of, and contact with, collaborators to form a steering group.
- Development and dissemination of surveys to the target audience to gather questions they have regarding PPID.
- Collation, categorisation and refining of the questions submitted by participants into a longlist, and formatting these collated questions in PICO (population, intervention, comparison, outcome) format where possible.

- Searching the literature to identify if the questions are 'uncertainties'. An uncertainty is defined by the JLA as "a question which cannot be answered by a relevant, up-to-date systematic review" (Petit-Zeman et al., 2010).

- If >30 uncertainties are identified, interim prioritisation is undertaken to form a short list of 25 questions taken forward to the PSP workshop.

The organisation of a PSP workshop

Establishing a Priority Setting Partnership

A steering group was established to run and oversee the Priority Setting Partnership (PSP). The steering group consisted of veterinary surgeons, including specialists in evidence-based medicine and equine internal medicine, as well as horse owners with experience of PPID. A protocol detailing the specific objectives of the PSP was developed in accordance with the JLA guide lines (Crowe, 2009; JLA, 2016) (Appendix 1) and adapted for use in an equine veterinary setting. The target population was veterinary surgeons and horse owners with experience of PPID. Boehringer Ingelheim Animal Health UK Limited (BI) was identified as a collaborator, and their extensive database of relevant participants facilitated survey dissemination.

Survey development and distribution

A survey was developed to collect questions from respondents based on the JLA guidelines using the freely available online survey tool, KwikSurveys (KwikSurveys, 2017) (Appendix 2). The survey gathered questions regarding the diagnosis, treatment and prognosis of PPID from veterinary surgeons and horse owners with experience of the disease. Open questions with free text boxes were used to facilitate this, and respondents could enter as many or as few questions as desired. An invitation to participate in the survey was distributed via email to collaborator BI's "Care and Connect" database (a service which enables owners and veterinary surgeons to monitor horses with PPID after diagnosis) (TAL, 2017) and BI's veterinary practice contact list: a tailored introduction was included explaining the purpose of the research. A link to the survey was also promoted through BI's Talk About Laminitis Facebook page, and the respective websites and/or social media pages of the University of Liverpool Equine Hospital, University of Nottingham Centre for Evidence Based Veterinary Medicine (CEBVM) and Animal Health Trust. The aim was to reach as many relevant participants as possible. Enrolment efforts were targeted within Great Britain (GB); however,

participation was not limited to respondents from GB. The survey was available online for eight weeks from 13th April 2017 to 9th June 2017.

Processing the responses

Responses were downloaded into a Microsoft Excel spreadsheet and anonymised. Each individual question was screened for relevance and to ensure the inclusion and exclusion detailed in the JLA PSP protocol (Appendix 1) were met. Questions outside the specific objectives of the PSP were excluded. Questions about diagnosis, treatment and prognosis of PPID were systematically categorised into themes. Duplicates and similar submissions were then interpreted and combined to create indicative questions to enable searching of the evidence base (Figure 1). For example, questions about the various possible side-effects of pergolide treatment were combined to form the CIRQ 'In horses with PPID, what are the side-effects of pergolide treatment both long and short-term?'. This enabled the themes and issues raised by the survey to be captured and made accessible to a non-research audience.

Verification of uncertainties

The literature was searched for relevant, up-to-date systematic reviews using appropriate databases including MEDLINE, CABI, SCOPUS, Web of Science (WOS) and the online database of citations for systematic reviews of relevance to veterinary medicine and science VetSRev (Grindlay et al., 2016). Broad search terms were used to ensure all relevant evidence was located (Appendix 3).The literature regarding diagnosis, treatment and prognosis of PPID was identified and categorised into type of study: [i] systematic review, [ii] narrative review, [iii] clinical trial, [iv] case-control study, [iv] observational study [prospective/retrospective], [v] cross-sectional study, [vi] descriptive reports and [vii] "other" (which included all other types of study including conference abstracts or where study design could not be determined).

Interim prioritisation

From the initial survey, more than 30 uncertainties were identified; therefore, an interim survey was undertaken to prioritise which uncertainties should be included in the PSP workshop (Appendix 4). A link to the interim survey was sent via email to all original survey respondents. To encourage additional responses, especially from veterinary surgeons, a link was also shared via the University of Liverpool Equine Practice Facebook page and other relevant social media pages replicating the original survey dissemination. This interim stage

required participants to select their top 10 most important questions from the longlist, but not to rank them. The interim prioritisation survey was available online from 23rd October 2017 to 20th November 2017. Questions were ranked via weighted scores, depending on the number of votes received, to ensure equal weighting of veterinary surgeon and horse owner responses. Separate rankings were then combined to form a final ranked list of all questions. The top 25 questions were taken forward to final PSP workshop.

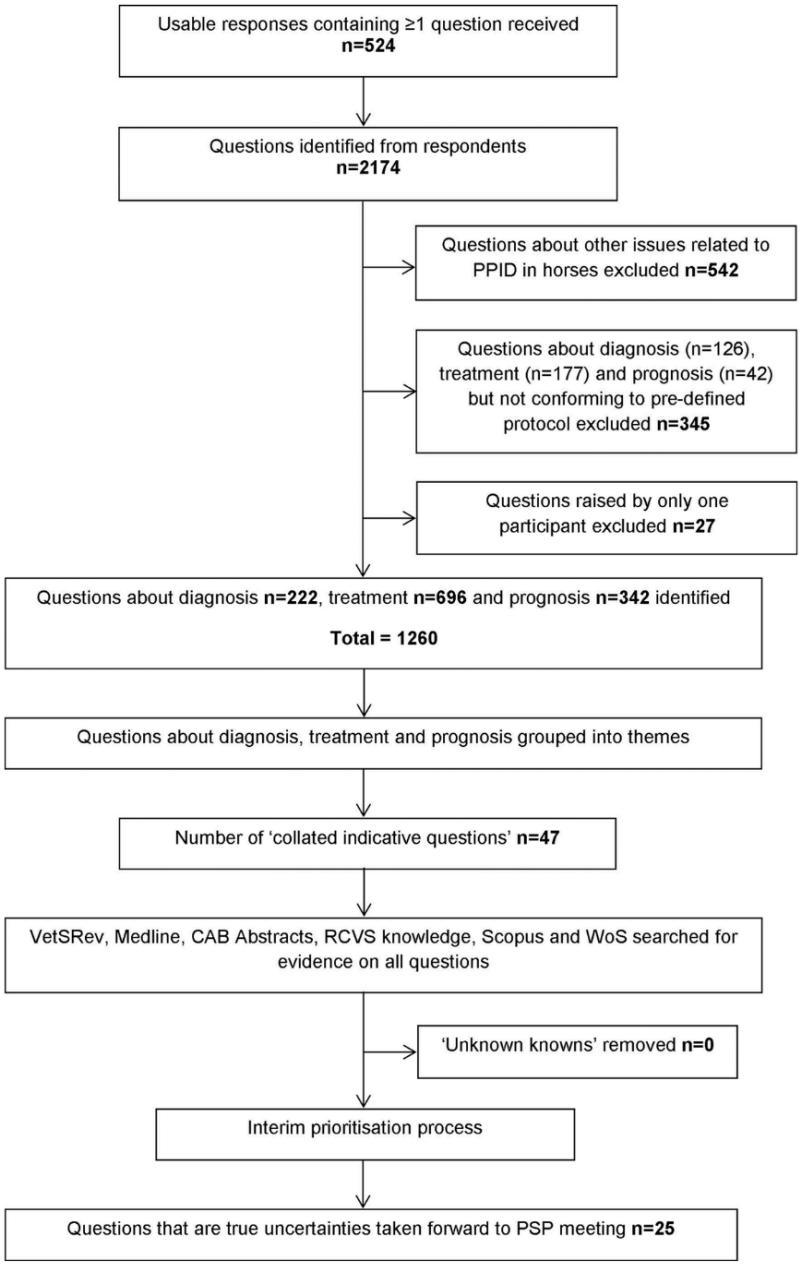


Figure 1: Process by which survey responses were converted to a shortlist of questions for prioritisation at the priority setting partnership workshop.

Priority setting workshop

Participants from both surveys who expressed an interest in participating further, and had provided contact details for this purpose, were invited to attend the final PSP workshop. The final priority setting was conducted in a face-to-face workshop using both small and whole group discussions, utilising an adapted nominal group technique (Potter et al., 2004; JLA, 2016). This allowed horse owners and veterinary surgeons to contribute equally to the discussion. The workshop was chaired and facilitated by the authors. To ensure transparency, each participant had to disclose who they were, what they did and any competing interests they had, for example if they worked in the pharmaceutical industry. Three rounds of prioritisation were undertaken using visual cue cards to rank questions; two small group sessions and one final whole group session. To ensure an even distribution of participants, veterinary surgeons and owners were assigned an identification number and randomly allocated to a group using a random number generator prior to the workshop (Augustana, 2004). The groups were purposively changed after the first session to ensure diverse collaboration and opinion sharing. After each round of prioritisation, the rankings from each small group were combined to form an overall aggregate rank for each question. The final ranking was then discussed and amended in the final whole group session with particular attention being paid to the top 10 collated questions.

Results

Respondent demographic information

A total of 524 usable responses, which contained at least one question about diagnosis, treatment and/or prognosis, were received from veterinary surgeons and/or horse owners with experience of PPID in the initial online survey. The majority of responses were from owners (92.6%; n=485), of which 438 currently owned a horse with PPID and 47 had previously owned a horse with PPID. The remaining 39 responses were from veterinary surgeons, of which 35 were currently in practice treating horses with PPID, and four were also current owners of a horse with PPID. The majority of respondents were from GB (92.4%; n=484), and 7.3% (n=38) were from other regions including North America (n=12), Europe (n=10), Ireland, the British Channel Islands and Isle of Man (n=9), Australasia (n=6) and Asia (n=1). A further two respondents did not provide information regarding their country of residence.

Collation and rationalisation of questions

The process of rationalising the list of questions is summarised in Figure 1. The 524 participants submitted a total of 2,174 individual questions about PPID in horses/ponies. The responses were very variable: some contained one question and others contained multiple questions. Some respondents did not submit a question at all and simply described their experiences or asked about their own horse specifically. These were utilised where relevant statements were made but otherwise excluded. Each response was broken down into possible questions and themes for the next step of the process, in which 1,260 questions specific to diagnosis, treatment and prognosis that met pre-defined criteria (Appendix 1) were identified. After thematic analysis, 47 CIRQs were identified.

Literature searches conducted on 06th December 2017 (Table 1 and Appendix 3) identified 134 relevant publications (after removal of duplicates), however no relevant up-to-date systematic reviews were identified (Table 1). Therefore, all 47 CIRQs from the survey were classed as unanswered, and consequently uncertainties, as defined by the JLA, and were taken forward to the two stage prioritisation process. After interim prioritisation, the 25 questions ranked highest overall were taken forward to the final PSP workshop (Table 2 and Appendix 5). Examples of the questions submitted by respondents, how they were categorised to form the CIRQs and their ranking following interim prioritisation are shown in Table 2.

Table 1: Study design and main topics of relevant publications regarding pituitary pars intermedia dysfunction identified via a literature search conducted on 06/12/2017 [n=134]

	Systematic Review	***Narrative Review**	***Clinical Trial**	***Case Control**	***Observational studies [prospective/ retrospective]**	***Cross-sectional**	***Descriptive reports**	***Other**
Diagnosis of PPID	0	12	0	20	9	3	26	14
Treatment of PPID	0	29	4	3	4	2	19	4
Prognosis of PPID	0	6	0	0	3	1	3	1

*Some studies covered multiple topics and therefore appear in the table more than once.

Table 2: The interim prioritisation rankings of the final top 10 questions agreed as shared priorities at the pituitary pars intermedia dysfunction priority setting partnership workshop, including examples of the original questions submitted and how they were categorised to form collated indicative research questions

Number of respondent questions which contributed to the final collated question	Example questions*	Collated indicative research question	Interim veterinary surgeon rank	Interim owner rank	Interim overall rank [veterinary surgeon and owner rankings combined]
45	• What is the likely progression of the disease or is it very individual. • How will it develop and what are the signs that it is developing? • Is there any way of assessing likely rates of deterioration of PPID?	In horses with PPID, what is the expected disease progression over a horse's lifetime both with and without treatment?	4	1	1
20	• Should the dosage vary at different times of year/when horse shows different symptoms? • I realise there are seasonal variations in the ACTH levels. Can the Prescend [*sic*] be safely reduced during off peak times? • And should the amount of medicine change through the seasons?	In horses with PPID, does the dose of pergolide need to vary with the season?	3	9	2

13	• By treating with Prascend, and lowering ACTH levels to within the normal range, how much will the likelihood of symptoms reduce? E.g. reduce laminitis risk by 70%, skin infections by 60% • Are they still at the same risk of laminitis as they were before treatment was started? • Is it true that if you reduce the prascend that you may cause laminitis?	In horses with PPID receiving treatment with pergolide, is the risk of laminitis reduced?	2	10	3
75	• Is there anything else that can be done to slow this disease apart from prascend? • If Prascend stops working what other treatments are available? • Can radiotherapy or surgery be used?	In horses with PPID, are there any medical treatments, other than pergolide, that work?	8	5	4
31	• Can results be inaccurate if a horse is stressed? • Can results be inaccurate if a horse is in pain? • Can other conditions such as ir and ems cause a false positive?	In horses with PPID, does stress, concurrent illness and/or pain affect the reliability and accuracy of diagnostic tests?	1	13	5
9	• Some cases refractory to treatment and difficult to explain why • What is the best way of treating apparent "non-responders" to pergolide? • What if treatment doesn't have the desired results?	What is the best way of dealing with horses who do not respond to pergolide treatment?	5	16	7

110	• What "lifestyle changes" should be made to help with the condition? • how can I keep my pony healthier while having PPID? • Are there any published studies of effective management and feed strategies for horses with PPID that can educate horse owners?	In horses with PPID, what additional management strategies [i.e. feed & turnout] are best to use in conjunction with pergolide treatment?	18	4	8
91	• Long term side effects of Prascend? • What are the side effects of the tablets? • Have you documented dysphagia as a side effect to prascend?	In horses with PPID, what are the side-effects of pergolide treatment [both long and short-term]?	17	7	9
3	• What is the recommended treatment/course of action after the horse has reached the recommended maximum daily number of Prascend tablets [i.e. 3 per day] and the ACTH levels are continuing to increase? • At what point in the progression of PPID is it advisable to discontinue increasing doses of pergolide? • What happens when you get to the maximum dose of prascend?	In horses with PPID, what should be done when the maximum dose of pergolide has been reached but hormone levels are still elevated?	16	12	11
67	• Homeopathic remedy that is as effective? • Have any herbal remedies ever been tested to work? • Are herbal/alternative treatments really effective in any way?	In horses with PPID, are any non-prescription treatments [i.e. Agnus Castus, homeopathy, other herbal products] effective?	37	2	16

11	• What is more important, clinical signs or bloods? What if the two don't match? • Should we diagnose an old horse without any clinical symptoms but a high ACTH in the blood, as having PPID? • What is the recommended plan for horses displaying symptoms of PPID but not testing positive?	In horses with suspected PPID, what is the best way to deal with inconclusive or conflicting test results and/or clinical signs [symptoms]?	10	35	21

*The raw questions are as they were entered by respondents, only spelling has been corrected.

The Priority Setting Partnership Workshop

The PSP workshop was attended by a total of nine veterinary surgeons and 13 horse owners with experience of PPID (Figure 2). A list of the top 10 uncertainties for PPID was agreed by consensus.

During the small group discussion rounds, each group decided on their shared list of priorities. Following the second round of small group discussions, there was little change overall in the order of the top eight questions. However, there was some change in the middle rankings. During the third whole group discussion, the final shared prioritised list of questions was agreed (Table 3). Overall, there was general consensus on the ranking for the majority of questions with the exception of question P ('In horses with PPID, are any non-prescription treatments (i.e. Agnus Castus, homeopathy, other herbal products) effective?'). There was much debate around this question and owners put forward a case for it to be moved into the top 10. However, agreement could not be reached on which question should be moved out of the top 10 in order to accommodate question P. Throughout the workshop discussions, participants considered that question P was closely related with question H ('In horses with PPID, what additional management strategies (i.e. feed & turnout) are best to use in conjunction with pergolide treatment?'). Therefore, it was decided that the two questions could be encompassed under a single ranking and that question P would form a 'part b' to question H (ranked fourth).

Following the PSP workshop, participants were sent a feedback questionnaire. The feedback questionnaire was completed by 16 attendees (five veterinary surgeons and eight owners, three did not specify). Median participant ratings for their overall experience, usefulness, purpose and organisation of the PSP workshop out of 10, were 9.5, 9, 10 and 10, respectively.

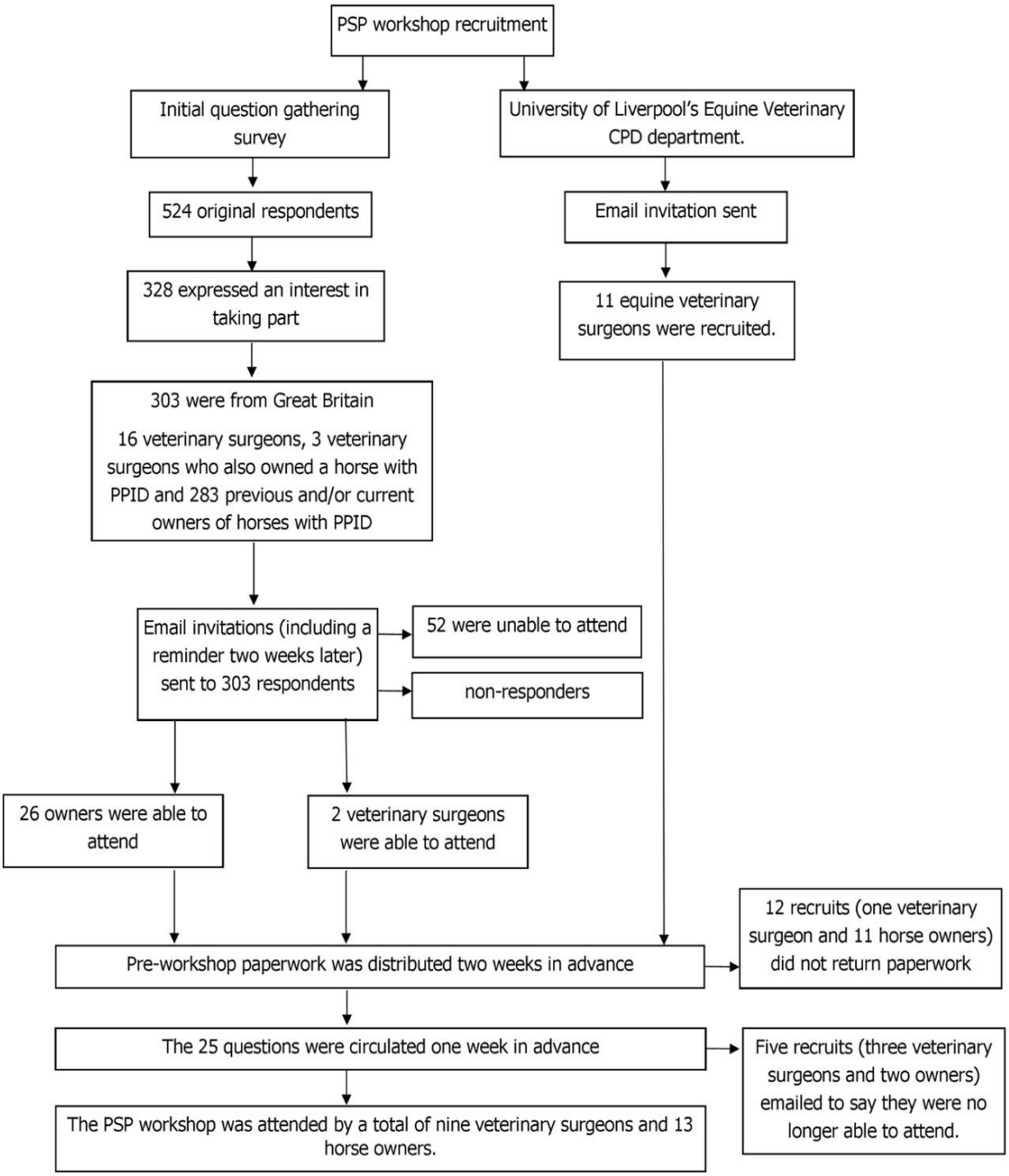


Figure 2: The process by which attendees were recruited to attend the PPID Priority Setting Partnership workshop.

Table 3: Final shared top ten research priorities for the diagnosis, treatment and prognosis of pituitary pars intermedia dysfunction (PPID) ranked during the PPID Priority Setting Partnership workshop

ID	Question	Final Order
A	In horses with PPID, what is the expected disease progression over a horse's lifetime both with and without treatment?	1
E	In horses with PPID, does stress, concurrent illness and/or pain affect the reliability and accuracy of diagnostic tests?	2
D	In horses with PPID, are there any medical treatments, other than pergolide, that work?	3
H	In horses with PPID, what additional management strategies [i.e. feed & turnout] are best to use in conjunction with pergolide treatment?	4a
P	In horses with PPID, are any non-prescription treatments [i.e. Agnus Castus, homeopathy, other herbal products] effective?	4b
C	In horses with PPID receiving treatment with pergolide, is the risk of laminitis reduced?	5
I	In horses with PPID, what are the side-effects of pergolide treatment [both long and short-term]?	6
B	In horses with PPID, does the dose of pergolide need to vary with the season?	7
U	In horses with suspected PPID, what is the best way to deal with inconclusive or conflicting test results and/or clinical signs [symptoms]?	8
G	What is the best way of dealing with horses who do not respond to pergolide treatment?	9
K	In horses with PPID, what should be done when the maximum dose of pergolide has been reached but hormone levels are still elevated?	10

Discussion

This PSP achieved engagement with horse owners and veterinary surgeons across two phases of prioritisation to identify research priorities for PPID. The JLA framework was successfully applied to an equine disease of complex pathophysiology, indicating this methodology could be effectively applied to other equine diseases. The research priorities, within the pre-defined topics of diagnosis, treatment and prognosis, included: [i] long-term prognosis, [ii] diagnostic accuracy, [iii] efficacy of pergolide, [iv] alternative treatment/management strategies and [v] potential treatment options for poor responders to treatment with pergolide. As the study was conducted in GB and respondents were primarily from GB, differences in horse populations, management and treatment practices around the world means the questions raised here may not be globally representative.

The PSP followed a rigorous and transparent predefined process to create a top 10 list of shared research priorities. The process gathered a large number of responses, comparable with other JLA PSPs (Eleftheriadou et al., 2011; Gadsby et al., 2012). The JLA does not define a target sample size. Instead, a saturation point is reached where no new themes are emerging (JLA, 2016). Participants posed multiple questions resulting in numerous themes and a large but manageable number of questions. At the point the survey was closed, no new themes were emerging.

Various methods were used to distribute the survey with some being more successful than others. Advertising via social media was effective for recruiting horse owners but not veterinary surgeons. A large number of veterinary surgeons were contacted via BI's mailing list. However, the number of veterinary responses compared to horse owners was disappointing. This may have been for a number of reasons, such as they were not interested in engaging in this type of research, the open question design of the questionnaire discouraged them from participating or they felt there were no gaps in the evidence and therefore did not have a question to pose. A more targeted approach or a prior, formal collaboration with veterinary practices may have improved veterinary responses. However, a low response rate from veterinary surgeons and veterinary practices has been noted in other studies (Hotchkiss et al. 2007a,b; Ireland et al., 2011a). Exploring motivators and barriers for veterinary involvement in research may improve future engagement.

Despite the low number of responses, questions posed by participating veterinary surgeons did not markedly differ from those posed by owners. Around a quarter of questions raised by participants were not specific to diagnosis, treatment or prognosis and instead covered other PPID-related subjects. This is comparable the previous veterinary PSP conducted by Dean, (2014). The majority of these non-useable questions were regarding the disease's pathophysiology which remains poorly understood (McFarlane, 2011). Many of the questions collated were un-structured and non-specific. Therefore, in order to form structured questions which enabled searching for evidence, questions were adapted and combined to form CIRQs. This stage was a qualitative process, with a set structure and technique set out by the JLA followed to ensure consistency (Crowe, 2009). This allowed questions to be combined and managed without losing context. A limitation of the process is that some of the research questions identified may represent a failure of communication, knowledge transfer or understanding, rather than actual evidence gaps. For example, submitted questions such as 'what diagnostic tools are available?' and 'what is it you are actually testing the levels of in the blood sample?' indicate that horse owners may not fully comprehend the diagnostic process. Therefore, the validity of each CIRQ was carefully considered by the steering group to ensure they were unanswered research questions, as defined by the JLA.

The 'Choose Ten' interim prioritisation approach was chosen because it is an uncomplicated way of allowing participants to consider the whole list then make choices that involve genuine shortlisting (JLA, 2016). Despite additional promotion, the number of responses from veterinary surgeons for the second interim survey remained low. The scoring and ranking method ensured that votes from veterinary surgeons and owners were equally weighted. A difference in priorities between groups was observed; some questions ranked highly by veterinary surgeons were ranked considerably lower by horse owners and vice versa. For example; 'In horses with PPID, does stress, concurrent illness and/or pain affect the reliability and accuracy of diagnostic tests?' was ranked highest by veterinary surgeons but thirteenth by owners.

During the adaptation of the JLA protocol, it was decided that the JLA definition of an 'uncertainty' would be applied for the equine veterinary setting. There are few systematic reviews in equine medicine; at the time of writing only 23 were available on the VetSRev database. This meant it was likely all questions posed by participants would be defined as uncertainties. However, it was agreed by the steering group that the level of certainty could

not be lowered and the quality of evidence should remain the same across fields. Systematic reviews collate data from numerous studies and offer the highest level of evidence (Howick et al., 2009), and if the JLA definition was changed it may have mislead PSP participants regarding the level of evidence available (Dean, 2014). The evidence base found for the diagnosis, treatment and prognosis of PPID was of poor to moderate quality (Howick et al., 2009). While no published systematic reviews pertaining to PPID were available prior to this PSP, equine endocrinology has been identified as a fast-moving field (Marr and Mair, 2014) and a large number of studies published within the past two decades contribute some evidence towards the top 10 research questions reported here. Future knowledge synthesis, such as systematic reviews, focused on these questions could offer an opportunity to close some of these evidence gaps. Importantly, this PSP has also identified a requirement to raise awareness within the equine veterinary profession of the need for better dissemination of the findings from previous research.

The final PSP workshop allowed an open and thoughtful exchange of views between horse owners and veterinary surgeons. This enabled consensus to be developed and facilitated the identification of the top 10 research questions. Both groups were represented by an appropriate number of participants (Crowe, 2009; JLA, 2016). All groups worked well together, with veterinary surgeons raising issues relating to owners and vice versa. As in other PSP workshops, changes in ranking after group discussions were noted (Finer et al., 2017). For example, the question 'In horses with PPID, how effective is pergolide at slowing the progression of the disease?' was ranked seventh and third by veterinary surgeons and owners respectively during the interim prioritisation, but was not prioritised into the final top 10. This is evidence of good group discussion and decision making, suggesting the ability to overcome biases (Finer et al., 2017). The number one prioritised question, 'In horses with PPID, what is the expected disease progression over a horse's lifetime both with and without treatment?' was ranked highly throughout the prioritisation process. Pituitary pars intermedia dysfunction is a chronic progressive disease associated with several comorbidities (McFarlane and Cribb, 2005; McFarlane, 2007; McGowan et al., 2013a; Bang and Frith, 2017) it is therefore unsurprising this question was ranked highest. It is a broad question encompassing several elements and therefore in the process of answering this question, it is possible a number of the other top 10 questions may also be answered. Several studies have investigated the initial efficacy of pergolide (Beech, 1994; Muñoz et al., 1996; van der Kolk, 1997; Donaldson et al., 2002; Perkins et al., 2002; Anon, 2011a). However, there is very little evidence regarding long-term effectiveness of treatment for improving prognosis.

One small study investigated treatment response after 5.5 years and found owners of surviving horses were satisfied with clinical response (Schott et al., 2014), and a recent retrospective study reported increased odds of short-term survival (median 11 months) in PPID cases treated with pergolide (Horn et al., 2019).

Two of the top 10 questions related to the accuracy and interpretation of diagnostic tests. In several studies, utilising a variety of different clinical reference standards, the commonly used basal ACTH test has been reported to have good sensitivity and specificity (Couëtil et al., 1996; Perkins et al., 2002; Horowitz et al., 2003; Frank et al., 2006a; Beech et al., 2007). However, uncertainty remains regarding test accuracy for PPID diagnosis in the presence of factors that may affect ACTH levels (Table 3, question E), such as concurrent disease (particularly insulin dysregulation), stress (Fazio et al., 2008) and pain (Ayala et al., 2012). In referral hospital populations, a number of acute conditions have been reported to result in elevations of ACTH (Ayala et al., 2012; Stewart et al., 2019). To date, these pre-analytical factors have not been evaluated in a population of PPID cases. However, the high proportion of systemically ill horses with ACTH concentrations above the upper limit of the reference interval at hospital admission (Stewart et al., 2019) indicates that it is important to consider these factors when interpreting basal plasma ACTH concentration for the diagnosis of PPID in practice. The second question encompassed respondents' uncertainly regarding the relative importance of endocrine laboratory test results and observed clinical signs. This included results that do not 'fit the clinical picture', such animals exhibiting clinical signs of PPID but with normal ACTH concentrations, as well as the interpretation of equivocal or 'borderline' test results and the best way to manage these situations (Table 2).

The majority of questions centred around treatment (Table 3), with questions focusing on additional management strategies, effective medical treatments other than pergolide [D], dosing of pergolide throughout the year [B], safety [I] and efficacy of pergolide treatment [C, G and K]. Several studies have reported that pergolide is effective at improving clinical signs and ACTH levels (Peters et al., 1995; Anon, 2011a; Schott et al., 2014), and this was generally the view point of participating veterinary surgeons at the beginning of the PSP. However, discussions with owners throughout the process highlighted that this is not always the case for individual animals. The process highlighted the need for a more robust evidence base for pergolide as a treatment and the need to investigate concurrent and alternative options.

Priority questions identified in this PSP are potentially methodologically complex to answer in terms of study design, implementation, ethical concerns and financial limitations. The JLA process is not concerned with how the questions raised will be answered: its function is to provide a platform for the involvement of end users (Elwyn et al., 2010; Petit-Zeman et al., 2010; JLA, 2017). However, the breadth of each topic offers researchers the opportunity to develop future studies dependent on resources available. Although all questions are considered important, it may not be possible to fund or answer all of them. In addition to supporting the direction of future research, the top 10 questions identify specific issues that horse owners consider important within each topic, providing a valuable resource to inform targeted owner education.

This study shows that horse owners and veterinary surgeons can be involved in identifying and prioritising uncertainties. The involvement of veterinary surgeons and owners at this stage of the research process has the ability to improve available evidence, ensure research is relevant to end users and aid decision making. During the PSP process horse owners acted as a proxy for the patient. The JLA has previously been utilised in this way for feline medicine (Dean, 2014). This adaptation is comparable to other JLA PSPs where patients cannot speak for themselves and carers or parents represent them, for example those involving children (Morris et al., 2015). The best interest of the patient remains the focus in each case.

Conclusion

The JLA methodology can be successfully adapted into an equine veterinary setting and applied to the diagnosis, treatment and prognosis of PPID in horses. The response and quantity of questions generated indicates an extensive number of uncertainties about the disease. However, as the research was undertaken in GB, it is possible that the research questions prioritised may differ from unanswered questions of veterinary surgeons and horse owners in other countries. Identifying the top 10 research questions for a disease or condition, especially those that require long-term management, will help to direct evidence synthesis, knowledge translation and future research into areas most important to the end users.

Declaration

This chapter was included with permission from the co-authors of the published version.

C. McGowan contributed to preparation and approval of the manuscript. R. Dean contributed to study design, data analysis and interpretation and approval of the manuscript. J. Ireland contributed to study design and preparation and approval of the manuscript.

Appendices for Chapter 2

Appendix 1: The Priority Setting Partnership Protocol

Pituitary Pars Intermedia Dysfunction Priority Setting Partnership

PROTOCOL 15/06/2017

Adapted from the James Lind Alliance protocol

Purpose of the PSP and background

The purpose of this protocol is to set out the aims, objectives and commitments of the Pituitary Pars Intermedia Dysfunction (PPID) Priority Setting Partnership (PSP) and the basic roles and responsibilities of the partners therein.

The Pituitary Pars Intermedia Dysfunction PSP will be led and managed by the primary investigator Becky Tatum BSc (Hons).

PSP lead and advisors (Steering Group)

Advisors to the student investigator will comprise of equine veterinary surgeons Dr Jo Ireland and Prof Cathy McGowan of the University of Liverpool, as well as horse owning members of the Animal Health Trust Epidemiology department, two of whom are also veterinary surgeons. Methodological guidance will be provided by veterinary surgeon and Director of the Centre of Evidence-based Veterinary Medicine (CEVM) Dr Rachel Dean. These representatives of horse owners and veterinary surgeons formed the steering group for this PSP.

Background to the Pituitary Pars Intermedia Dysfunction Priority Setting Partnership

PPID is the most common endocrine disorder in older horses, affecting 21% of those aged 15 years or older and is therefore of significant importance in equine practice. It is a progressive endocrine disease which causes abnormal coat changes and fat distribution among other clinical signs, as well as comorbidities such as laminitis. The available evidence regarding the diagnosis, treatment and prognosis of PPID is limited both in terms of the study populations included and the outcomes measured. An evidence base is needed to inform veterinary surgeons and owners, yet the quality of published evidence is fair to poor. Therefore, it is important that any research done in this area is applicable to practice and can be used directly by those that can improve patient care. The evidence gaps in the diagnosis, treatment and prognosis of PPID need to be identified and prioritised.

Aims and objectives of the Pituitary Pars Intermedia Dysfunction (PPID) PSP

The aim of the PPID PSP is to identify the unanswered questions about PPID diagnosis, treatment and prognosis from horse owner and clinical perspectives, then prioritise those that horse owners and veterinary surgeons agree are the most important. A secondary aim is to ascertain if the JLA PSP framework can be applied to equine veterinary practice.

The objectives of the PPID PSP are to:

- Work with horse owners and veterinary surgeons to identify uncertainties about the accuracy, reliability and effectiveness of diagnosis techniques, including screening for PPID.
- Work with horse owners and veterinary surgeons to identify uncertainties about the efficacy and effectiveness of PPID treatments, including monitoring and response to treatment.
- Work with horse owners and veterinary surgeons to identify uncertainties about the prognosis of horses and ponies with PPID, both long and short term including quality of life and influencing factors.
- To agree by consensus a prioritised list of those uncertainties, for research purposes
- To publicise the results of the PSP and process
- To take the results to research commissioning bodies to be considered for funding.

Partners

Organisations and individuals will be invited to be involved with the PSP as partners. Partners are groups or individuals who will commit to supporting the PSP by disseminating the PSP survey. Partners represent the following groups:

- owner of horses with experience of PPID
- veterinary surgeons with clinical experience of PPID

It is important that organisations which can reach and advocate for these groups should be invited to become involved in the PSP. Boehringer Ingelheim Vetmedica Ltd (BI) has been engaged to collaborate with the project as they have contacts for both horse owners and veterinary surgeons with experience of PPID. To ensure wider coverage the Universities of Liverpool and Nottingham, Animal Health Trust and Veteran Horse Society were also engaged to collaborate.

Some organisations may be judged as having conflicts of interest. These can be perceived to adversely affect those organisations' views, causing unacceptable bias. This is likely to affect the ultimate findings of the PSP. As a pharmaceutical company BI is considered to have a conflict of interest. However, the Steering Group considers their partnership important to target a large number of relevant participants; therefore, BI will participate in a purely observational capacity once the survey has been disseminated.

Methods

This section describes a schedule of proposed stages through which the PSP aims to fulfil its objectives. The process is iterative and dependent on the active participation and contribution of different groups. The methods adopted in any stage will be agreed through consultation between the steering group members, guided by the PSP's aims and objectives. More details can be found in the Guidebook section of the JLA website at www.jla.nihr.ac.uk where examples of the work of other JLA PSPs can also be seen.

Step 1: Identification and invitation of potential partners and raising stakeholder awareness

Potential partner organisations will be identified through a process of peer knowledge and consultation, through the Steering Group members' networks. Potential partners will be contacted and informed of the establishment and aims of the PPID PSP.

Step 2: Identifying uncertainties

An online survey will be developed to identify questions veterinary surgeons and horse owners have about PPID. The survey will be based on the guidelines outlined in the JLA guidebook and adapted through consultation with the steering group. BI, the Animal Health Trust, Veteran Horse Society and the Universities of Liverpool and Nottingham will identify a method for distributing the initial survey to potential participants. This will enable questions and uncertainties of practical clinical importance relating to the diagnosis, treatment and prognosis of PPID to be identified. A period of 6 - 8 weeks will be given to complete this exercise or until no new themes are emerging from survey results.

Step 3: Refining questions and uncertainties

The consultation process (survey) will produce "raw" unanswered questions about diagnosis, treatments and prognosis. These raw questions will be assembled and categorised and refined by Becky Tatum into "collated indicative questions" which are clear, addressable by research and understandable to all.

Questions will undergo two initial rounds of refinement, firstly to remove questions not relating to diagnosis, treatment or prognosis. Such as prevalence, prevention, risk factors, pathophysiology and associated diseases.

Secondly out-of-scope submissions about diagnosis, treatment and prognosis which do not fall under the specific aims and objectives of the study will be removed. This will include, for example, questions relating to understanding diagnostic test results, availability of treatment options/how PPID is treated or whether there is a 'cure' for the disease.

Relevant statements which can be formatted or merged into questions will be included where appropriate.

Relevant submissions will be further refined by removing:

- Questions submitted by only one participant
- Incomplete submissions which do not define the respondent's characteristics i.e. whether they are a veterinary surgeon or horse owner with experience of PPID (this information is needed to ensure the transparency of the refining process)
- Submissions from respondents that do not have experience of PPID

Categorisation and thematic analysis will be used to identify similar or duplicate questions which will be combined where appropriate. Questions will then be reformatted and reworded where necessary to form research questions understandable to all.

Existing sources of information about uncertainties for owners and clinicians will be searched. This will include research recommendations in systematic reviews and other evidence; clinical trials, narrative reviews and clinical guidelines. To identify sources of uncertainties and research recommendations Medline, Web of Science, Vetsrev, Scopus, and CAB databases will be searched. The existing literature will be researched by Becky Tatum, with the assistance of Dr Jo Ireland and Prof Cathy McGowan, to see to what extent these refined questions have, or have not, been answered by previous research.

If questions are expressed can be answered with reference to existing research evidence (systematic reviews that meet the JLA criteria of certainty) and are therefore "unknown knowns" and not uncertainties, this evidence will be highlighted by the PSP to their membership. This suggests that information is not being communicated effectively to those who need it. A separate record of these 'answerable questions' will be kept.

Uncertainties that are not adequately addressed by previous research will be collated for interim (if applicable) and final prioritisation. The checking undertaken to demonstrate that the uncertainties have not already been answered will be recorded.

Steps 4 and 5: Prioritisation – interim and final stages

The aim of the final stage of the priority setting process is to prioritise through consensus, the identified uncertainties relating to the diagnosis, treatment or prognosis of PPID. This will be carried out by members of the Steering Group and the wider partnership that represents horse owners and veterinary surgeons.

The interim stage

The refining process will result in a long list of indicative uncertainties, the number of which is hard to predict. If the long list of indicative uncertainties is >30 it will be reduced to a shortlist to be taken forward and discussed at the final PSP workshop. This will be achieved via a consultation process carried out over email to the steering group and participants who completed the survey and agreed

to partake in the next stages of the study. Participants in this ranking process will be invited to choose 10 uncertainties from the long (or interim) list. They will not be asked to prioritise them. The responses obtained will be used to rank the uncertainties by number of votes. The top 25 will be taken forward into the final PSP workshop.

The final stage

The process to reach 10 prioritised uncertainties will be conducted in a face-to-face workshop, using group discussions and plenary sessions. This will involve eligible members of the Steering Group and the wider partnership that represents horse owners and veterinary surgeons. The method used for this prioritisation process will be an adapted nominal group technique overseen by Becky Tatum and Dr Rachel Dean to ensure equal contribution from participants.

The aim is to hold one workshop including questions about diagnosis, treatment and prognosis. However, if the number of questions regarding these individual areas is deemed by the Steering Group to warrant three separate PSP workshops, treatment uncertainties will be prioritised in the face-to-face workshop while uncertainties about diagnosis and prognosis will be prioritised online using adapted Delphi technique.

Findings, research and publicity

Findings and research

It is anticipated that the findings of the PPID PSP will be reported to the pharmaceutical industry and major research funding charities as well as other funding bodies for clinical veterinary research.

Relevant Steering Group members and partners will publish the 'top 10' priorities on their websites and in various publications to horse owners and the veterinary profession. All partners will be encouraged to develop the prioritised uncertainties into research questions, and to work to establish the research needs of those unanswered questions to use when approaching potential funders, or when allocating funding for research themselves, if applicable.

As well as alerting funders, partners and advisory group members the results will be published using both internal and external communication mechanisms. This will be done in an open access format that is easy to understand to all participants and potentially interested funders of research. The

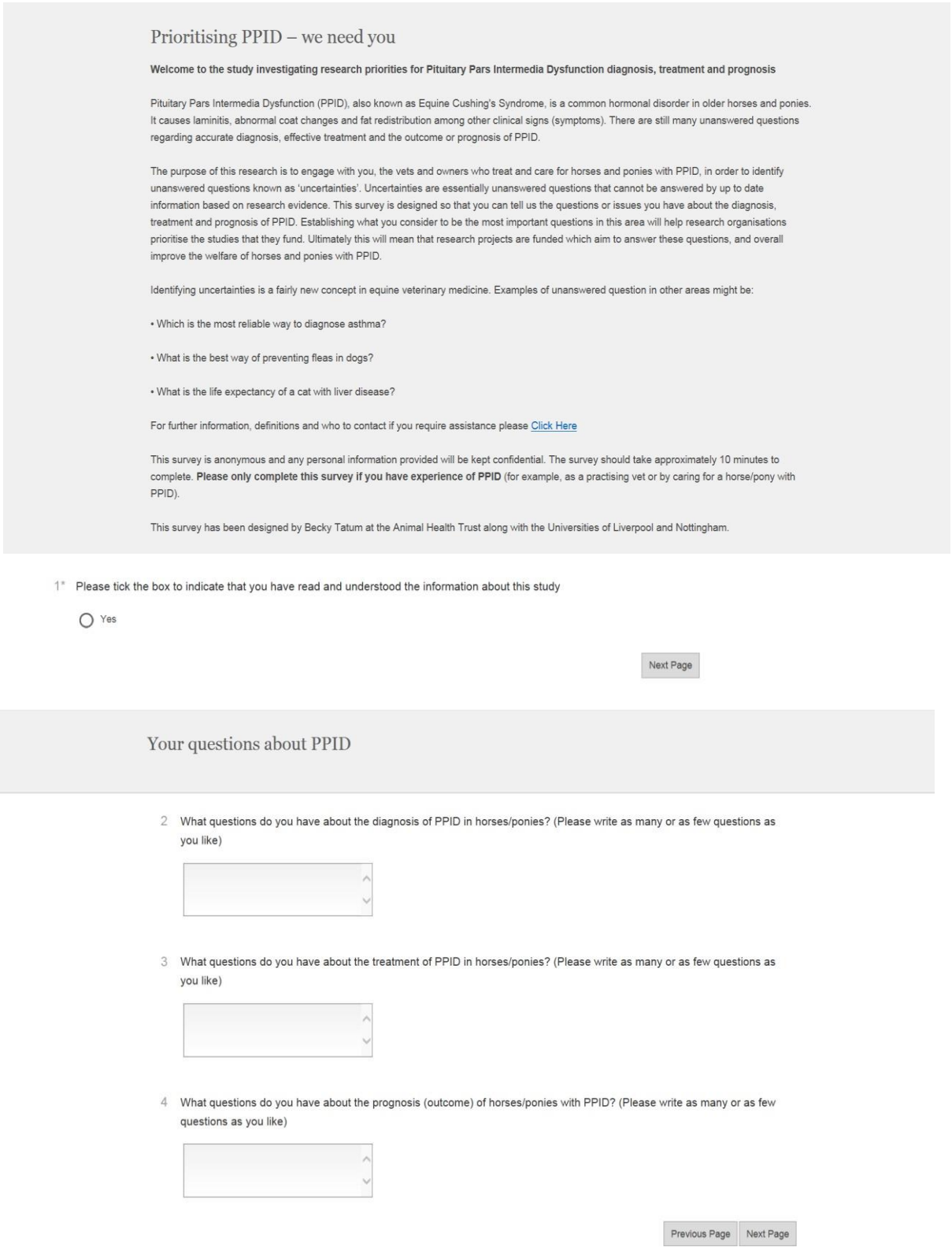
findings will also be the topic of an MPhil thesis and peer reviewed papers. The production of an academic paper should not take precedence over publicising of the final results.

Signed by the Primary Investigator

The undersigned agree to follow the PPID Priority Setting Protocol.

Becky Tatum 15/06/2017

Appendix 2: The uncertainty gathering survey.



Prioritising PPID – we need you

Welcome to the study investigating research priorities for Pituitary Pars Intermedia Dysfunction diagnosis, treatment and prognosis

Pituitary Pars Intermedia Dysfunction (PPID), also known as Equine Cushing's Syndrome, is a common hormonal disorder in older horses and ponies. It causes laminitis, abnormal coat changes and fat redistribution among other clinical signs (symptoms). There are still many unanswered questions regarding accurate diagnosis, effective treatment and the outcome or prognosis of PPID.

The purpose of this research is to engage with you, the vets and owners who treat and care for horses and ponies with PPID, in order to identify unanswered questions known as 'uncertainties'. Uncertainties are essentially unanswered questions that cannot be answered by up to date information based on research evidence. This survey is designed so that you can tell us the questions or issues you have about the diagnosis, treatment and prognosis of PPID. Establishing what you consider to be the most important questions in this area will help research organisations prioritise the studies that they fund. Ultimately this will mean that research projects are funded which aim to answer these questions, and overall improve the welfare of horses and ponies with PPID.

Identifying uncertainties is a fairly new concept in equine veterinary medicine. Examples of unanswered question in other areas might be:

• Which is the most reliable way to diagnose asthma?

• What is the best way of preventing fleas in dogs?

• What is the life expectancy of a cat with liver disease?

For further information, definitions and who to contact if you require assistance please Click Here

This survey is anonymous and any personal information provided will be kept confidential. The survey should take approximately 10 minutes to complete. **Please only complete this survey if you have experience of PPID** (for example, as a practising vet or by caring for a horse/pony with PPID).

This survey has been designed by Becky Tatum at the Animal Health Trust along with the Universities of Liverpool and Nottingham.

1* Please tick the box to indicate that you have read and understood the information about this study

○ Yes

Next Page

Your questions about PPID

2 What questions do you have about the diagnosis of PPID in horses/ponies? (Please write as many or as few questions as you like)

3 What questions do you have about the treatment of PPID in horses/ponies? (Please write as many or as few questions as you like)

4 What questions do you have about the prognosis (outcome) of horses/ponies with PPID? (Please write as many or as few questions as you like)

Previous Page Next Page

Treating PPID

If you do not currently own or care for a horse/pony diagnosed with PPID please continue to question 8 on the next page

5 If you currently own or care for a horse/pony with PPID, how long ago were they diagnosed?

- ◯ Within the last 12 months
- ◯ 1-3 years ago
- ◯ Over 3 years ago

6 Did your horse/pony receive any medical treatment within the first 12 months after their diagnosis?

- ◯ Yes, received Pergolide
- ◯ No, did not receive treatment
- ◯ Yes, Other (Please Specify)

7 If your horse/pony was diagnosed more than 12 months ago, are they still receiving medical treatment?

- ◯ Yes, currently receiving Pergolide
- ◯ No, not currently receiving treatment
- ◯ Other (Please Specify)

Previous Page | Next Page

About you

8* Please select the option which best describes you

- ◯ I am a veterinary surgeon who treats horses and ponies with PPID
- ◯ I am an owner/carer who currently cares for a horse or pony with PPID
- ◯ I am an owner/carer who has previously cared for a horse or pony with PPID
- ◯ Other (Please Specify)

9 How did you find out about this study?

- ☐ Via Care and Connect
- ☐ Via the Animal Health Trust
- ☐ Via the Veteran Horse Society
- ☐ Directly from my veterinary surgeon
- ☐ Through a friend/colleague/client
- ☐ Via other social media

Other (Please Specify)

10 Where do you currently live?

- ◯ Within Great Britain
- ◯ Outside Great Britain (please specify where)

11* As part of this research we may publish the uncertainty/question that you, along with other participants, have identified. When reporting the results, no participants will be identified and your participation in this study will remain entirely anonymous. Please indicate if we have your permission to do so

- ◯ Yes
- ◯ No

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The next stage of the study

Once responses from owners and veterinary surgeons have been collected, volunteers will be needed to help us prioritise which of the questions identified are the most important. This will be done at a meeting in either the Newmarket or Liverpool area (depending on which is most convenient). The meeting will involve both owners and veterinary surgeons with experience of PPID who have participated in this survey. You do not need experience of participating in a meeting like this before to take part, and all contributions will be highly valued. Unfortunately limited funding means we are unable to pay participants for attending this meeting, however refreshments will be provided (for participating veterinary surgeons the meeting will count as CPD).

12 If you are interested in being involved in the next stage of this study please complete your contact details below. All personal information will be treated confidentially

Name

Address 1

Address 2

Town/City

County

Postcode

Contact phone number

Email address

13 If you would like to be contacted with the results of the study please provide your email address below

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Thank you for completing this survey.

Your contribution to our research is appreciated.

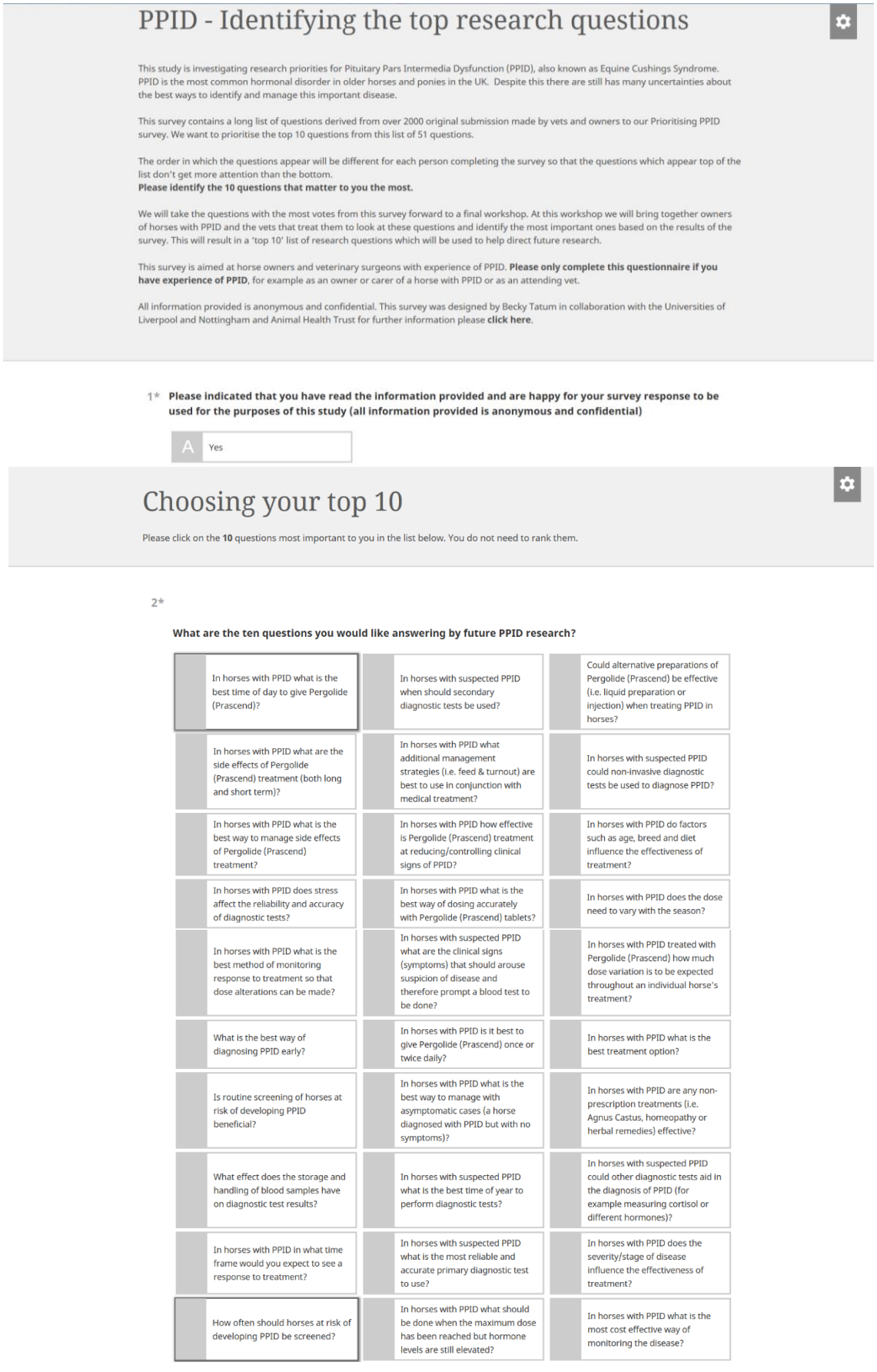
If you would like any more information please contact Becky Tatum at the Animal Health Trust: becky.tatum@aht.org.uk

Appendix 3: Search strategy

Details of search terms used and databases searched when evaluating evidence for the diagnosis, treatment and prognosis of pituitary pars intermedia dysfunction

Databases searched	Medical Literature Analysis and Retrieval System Online (MEDLINE) 1966-2017 Centre for Agriculture and Bioscience International (CABI) 1972-2017 Elsevier's abstract and citation database (SCOPUS) 1966-2017 Web of Science (WOS) 1945-2017	Via University of Liverpool library
	VetSRev	Via Centre for Evidence-Based Veterinary Medicine
Search terms:	Horse* OR pony OR ponies OR equine* OR equid* AND Pituitary pars intermedia dysfunction OR PPID OR cushing* OR pituitary neoplasia OR hyperadrenocorticism OR pituitary adenoma*	
Dates searches performed:	06/12/2017	

Appendix 4: The interim prioritisation survey



PPID - Identifying the top research questions

This study is investigating research priorities for Pituitary Pars Intermedia Dysfunction (PPID), also known as Equine Cushings Syndrome. PPID is the most common hormonal disorder in older horses and ponies in the UK. Despite this there are still has many uncertainties about the best ways to identify and manage this important disease.

This survey contains a long list of questions derived from over 2000 original submission made by vets and owners to our Prioritising PPID survey. We want to prioritise the top 10 questions from this list of 51 questions.

The order in which the questions appear will be different for each person completing the survey so that the questions which appear top of the list don't get more attention than the bottom.
Please identify the 10 questions that matter to you the most.

We will take the questions with the most votes from this survey forward to a final workshop. At this workshop we will bring together owners of horses with PPID and the vets that treat them to look at these questions and identify the most important ones based on the results of the survey. This will result in a 'top 10' list of research questions which will be used to help direct future research.

This survey is aimed at horse owners and veterinary surgeons with experience of PPID. **Please only complete this questionnaire if you have experience of PPID**, for example as an owner or carer of a horse with PPID or as an attending vet.

All information provided is anonymous and confidential. This survey was designed by Becky Tatum in collaboration with the Universities of Liverpool and Nottingham and Animal Health Trust for further information please **click here**.

1* **Please indicated that you have read the information provided and are happy for your survey response to be used for the purposes of this study (all information provided is anonymous and confidential)**

A Yes

Choosing your top 10

Please click on the **10** questions most important to you in the list below. You do not need to rank them.

2*

What are the ten questions you would like answering by future PPID research?

In horses with PPID what is the best time of day to give Pergolide (Prascend)?	In horses with suspected PPID when should secondary diagnostic tests be used?	Could alternative preparations of Pergolide (Prascend) be effective (i.e. liquid preparation or injection) when treating PPID in horses?
In horses with PPID what are the side effects of Pergolide (Prascend) treatment (both long and short term)?	In horses with PPID what additional management strategies (i.e. feed & turnout) are best to use in conjunction with medical treatment?	In horses with suspected PPID could non-invasive diagnostic tests be used to diagnose PPID?
In horses with PPID what is the best way to manage side effects of Pergolide (Prascend) treatment?	In horses with PPID how effective is Pergolide (Prascend) treatment at reducing/controlling clinical signs of PPID?	In horses with PPID do factors such as age, breed and diet influence the effectiveness of treatment?
In horses with PPID does stress affect the reliability and accuracy of diagnostic tests?	In horses with PPID what is the best way of dosing accurately with Pergolide (Prascend) tablets?	In horses with PPID does the dose need to vary with the season?
In horses with PPID what is the best method of monitoring response to treatment so that dose alterations can be made?	In horses with suspected PPID what are the clinical signs (symptoms) that should arouse suspicion of disease and therefore prompt a blood test to be done?	In horses with PPID treated with Pergolide (Prascend) how much dose variation is to be expected throughout an individual horse's treatment?
What is the best way of diagnosing PPID early?	In horses with PPID is it best to give Pergolide (Prascend) once or twice daily?	In horses with PPID what is the best treatment option?
Is routine screening of horses at risk of developing PPID beneficial?	In horses with PPID what is the best way to manage with asymptomatic cases (a horse diagnosed with PPID but with no symptoms)?	In horses with PPID are any non-prescription treatments (i.e. Agnus Castus, homeopathy or herbal remedies) effective?
What effect does the storage and handling of blood samples have on diagnostic test results?	In horses with suspected PPID what is the best time of year to perform diagnostic tests?	In horses with suspected PPID could other diagnostic tests aid in the diagnosis of PPID (for example measuring cortisol or different hormones)?
In horses with PPID in what time frame would you expect to see a response to treatment?	In horses with suspected PPID what is the most reliable and accurate primary diagnostic test to use?	In horses with PPID does the severity/stage of disease influence the effectiveness of treatment?
How often should horses at risk of developing PPID be screened?	In horses with PPID what should be done when the maximum dose has been reached but hormone levels are still elevated?	In horses with PPID what is the most cost effective way of monitoring the disease?

What is the prognosis for horses with PPID? Compared to horses without the disease?	In horses with PPID does concurrent illness and/or pain affect the reliability and accuracy of diagnostic tests?	In horses with PPID does treatment affect the prognosis?
In horses with PPID what is the expected disease progression over the horse's lifetime both with and without treatment?	In horses with PPID starting Pergolide (Prascend) treatment what is the best way to avoid initial side effects?	In horses with suspected PPID can a definitive diagnosis be made on clinical signs (symptoms) alone?
In horses with PPID receiving treatment with Pergolide (Prascend) is the risk of laminitis reduced?	In horses with PPID are there consequences if Pergolide (Prascend) doses are missed, if so what are they?	In horses with PPID what is the best way to manage borderline cases?
In horses with PPID are there any other medical treatments that work?	What is the best way of dealing with horses who do not respond to Pergolide (Prascend) treatment?	In horses with PPID do factors such as diet, management or time of day affect the reliability and accuracy of diagnostic tests?
In horses with suspected PPID what is the best way to deal with inconclusive or conflicting test results/clinical signs?	In horses with PPID how do we improve prognosis?	In horses with PPID how effective is Pergolide (Prascend) at slowing the progression of the disease?
In horses with PPID how long is Pergolide (Prascend) treatment effective for?	In horses with suspected PPID is a single diagnostic test sufficient to diagnose PPID, or is re-testing required to provide a conclusive diagnosis?	In horses with PPID do factors such as age, breed or management affect prognosis?
In horses with PPID what are the long term effects of the disease?	In horses with PPID does concurrent illness affect prognosis?	In horses with PPID does the stage/severity at diagnosis affect prognosis?

About you

3* **Did you take part in the initial Prioritising PPID survey?**

A	Yes	B	No	C	Don't Know

4* **Which of the following best describes you?**

A	I am a veterinary surgeon who treats horses and ponies with PPID	B	I am an owner/carer who currently cares for a horse or pony with PPID	C	I am an owner/carer who previously cared for a horse or pony with PPID
D	Both a veterinary surgeon and owner/carer of a horse or pony with PPID	E	Both a previous and current owner/carer of a horse or pony with PPID		
	Other (Please Specify)				

5 **How did you find out about this study?**

A	Via the Talk about laminitis (TAL) scheme	B	Via the Animal Health Trust	C	Directly from your veterinary surgeon
D	Through a friend/colleague/client	E	Via social media	F	Via the University of Liverpool
	Other (Please Specify)				

6 **Where do you currently live?**

A	Within Great Britain	B	Outside Great Britain
	If you currently live outside Great Britain please specify where		

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The final stage of the study

The final stage of the study involves a workshop where vets and owners will come together to prioritise the questions with the most votes from this survey into a list of the 'top 10' most important research questions which need answering. The resulting top 10 will help direct future PPID research into the areas most important and useful to you, the end users. The workshop will be held in the Liverpool area on 8th December, you do not need experience of this kind of meeting to attend and all contributions are highly valued. Travel expenses will be reimbursed and for vets it counts as CPD.

7

If you would like to take part in the prioritisation workshop please complete you contact details below. All personal information will be treated confidentially

Name

Address

Address

Town

County

Postcode

Email Address

Phone Number

8

If you would like to be informed of the results of this study please provide your email address below

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Thank you for completing this survey and contributing towards our research.

If you would like any further information please contact Becky Tatum at becky.tatum@aht.org.uk

Appendix 5: The 25 questions ranked highest overall after the interim prioritisation and taken forwards to the priority setting partnership.

Question ID	Question
A	In horses with PPID, what is the expected disease progression over a horse's lifetime both with and without treatment?
B	In horses with PPID, does the dose of pergolide (Prascend) need to vary with the season?
C	In horses with PPID receiving treatment with pergolide (Prascend), is the risk of laminitis reduced?
D	In horses with PPID, are there any medical treatments, other than pergolide (Prascend), that work?
E	In horses with PPID, does stress, concurrent illness and/or pain affect the reliability and accuracy of diagnostic tests?
F	In horses with PPID, how effective is pergolide (Prascend) at slowing the progression of the disease?
G	What is the best way of dealing with horses who do not respond to pergolide (Prascend) treatment?
H	In horses with PPID, what additional management strategies (i.e. feed & turnout) are best to use in conjunction with pergolide (Prascend) treatment?
I	In horses with PPID, what are the side effects of pergolide (Prascend) treatment (both long and short term)?

J	In horses with PPID, what is the best method of monitoring response to treatment so that dose alterations can be made?
K	In horses with PPID, what should be done when the maximum dose of pergolide (Prascend) has been reached but hormone levels are still elevated?
L	What is the prognosis for horses with PPID?
M	In horses with PPID, what is the best way to manage asymptomatic cases (a horse diagnosed with PPID but with no symptoms)?
N	In horses with PPID, do co-existing illnesses (such as equine metabolic syndrome, arthritis or laminitis) affect prognosis?
O	In horses with PPID, what is the best way to manage side effects of pergolide (Prascend) treatment?
P	In horses with PPID, are any non-prescription treatments (i.e. Agnus Castus, homeopathy, other herbal products) effective?
Q	In horses with PPID, how do we improve prognosis?
R	Could alternative preparations of pergolide (Prascend) be effective (i.e. liquid preparation or injection) when treating PPID in horses?
S	In horses with PPID, what is the best way to manage borderline cases?
T	In horses with PPID, how effective is pergolide (Prascend) treatment at reducing/controlling clinical signs (symptoms) of PPID?
U	In horses with suspected PPID, what is the best way to deal with inconclusive or conflicting test results and/or clinical signs (symptoms)?

V	In horses with PPID, do factors such as diet, management or time of day affect the reliability and accuracy of diagnostic tests?
W	In horses with PPID, what is the most cost effective way of monitoring the disease?
X	In horses with PPID, how long is pergolide (Prascend) treatment effective for?
Y	In horses with PPID, what are the long term effects of the disease?

Chapter 3

Evaluation of the sensitivity and specificity of basal plasma adrenocorticotrophic hormone concentration for diagnosing pituitary pars intermedia dysfunction in horses: A systematic review

Evaluation of the sensitivity and specificity of basal plasma adrenocorticotrophic hormone concentration for diagnosing pituitary pars intermedia dysfunction in horses: a systematic review

The following chapter collates and reviews literature pertaining the diagnostic accuracy of the basal plasma adrenocorticotrophic hormone concentration test for diagnosis of PPID and is presented as it was published in the Veterinary Journal.

Reference: Tatum RC, McGowan CM, Ireland JL (2021) Evaluation of the sensitivity and specificity of basal plasma adrenocorticotrophic hormone concentration for diagnosing pituitary pars intermedia dysfunction in horses: a systematic review. The Veterinary Journal 275: 105695.

Summary

Measurement of basal adrenocorticotrophic hormone (ACTH) is currently considered to possess adequate accuracy for the diagnosis of pituitary pars intermedia dysfunction (PPID) in horses. However, there is a lack of a 'gold standard' diagnostic test and although recommendations have been published, a systematic review of the evidence for its use has not been undertaken. This study aimed to systematically review the current evidence regarding the sensitivity and specificity of the basal ACTH diagnostic test. Electronic databases were systematically searched in January 2019, September 2020 and updated in January 2021. English language publications published prior to these dates were included. Screening, data extraction and quality assessment of publications was undertaken individually by the authors using predefined criteria and subsequently cross-checked. Study design, methodology and information reported in included studies were assessed using STARD checklists. Risk of bias and applicability were appraised using the QUADAS-2 quality assessment tool for diagnostic accuracy studies. Due to identified biases and marked between-study variations, meta-analysis was not undertaken.

After removal of duplicates, 415 publications were identified, of which 25 publications were evaluated in full, with 11 of these publications meeting inclusion criteria. In the majority of studies, the basal ACTH test was reported to have good sensitivity (overall median 75.5%; interquartile range (IQR) 64.0-86.5%; range 36.0-100%) and excellent specificity (overall median 95.2%; IQR 84.2-98.9%; range 63.3-100%). However, QUADAS-2 and STARD assessment highlighted that studies did not utilise optimal study design and/or study

populations for the evaluation of a diagnostic test and the majority were subject to bias or provided insufficient information to fully assess possible biases. The evidence base needs to be improved by utilising a representative sample of cases suspected of having PPID, a diagnostic cut-off value defined *a priori* and an appropriate reference standard, before any concrete recommendations can be made, or conclusions drawn. Based on this review, basal ACTH performed better at ruling out PPID than detecting it.

Introduction

Pituitary pars intermedia dysfunction (PPID) is a progressive neurodegenerative disease, common in older equids (McGowan et al., 2013a). A loss of dopaminergic inhibitory output to the pars intermedia of the pituitary gland results in increased plasma concentrations of multiple pro-opiomelanocortin (POMC) derived peptides, including adrenocorticotrophic hormone (ACTH) (van der Kolk, 1997; McFarlane et al., 2005b). This leads to a spectrum of clinical signs including lethargy or depression-like state, coat abnormalities and muscle atrophy (Ireland and McGowan, 2018). Historically, diagnosis was predominantly based on age and evidence of hypertrichosis, which may be adequate for detection of advanced stages of disease (Schott, 2002; Frank et al., 2006a). However, clinical diagnosis is more challenging in the earlier stages of disease when classical signs may not be evident or in cases with less specific clinical signs (Miller et al., 2008). Therefore, a number of different endocrine laboratory tests have been developed, evaluated and used for the diagnosis of PPID. These include basal tests such plasma ACTH and α-melanocyte stimulating hormone (α-MSH) concentrations (McFarlane et al., 2006; McGowan et al., 2013b), and dynamic tests including overnight dexamethasone suppression (ODST) and thyrotropin-releasing hormone (TRH) stimulation tests (Frank et al., 2006a; Beech et al., 2007).

Anecdotally the diagnostic test of choice in the UK is basal ACTH concentration. This is due to the convenience of collecting a single blood sample, the wide availability of diagnostic assays and seasonal reference intervals that allow year round testing (Copas and Durham, 2012; Rendle et al., 2015a; Durham et al., 2020). This blood test is currently considered to possess adequate accuracy for clinical use (Durham et al., 2014a). However, there is a lack of a 'gold standard' laboratory test (McGowan et al., 2013b) and although diagnostic recommendations have been published (EEG, 2019; Secombe et al., 2018), a systematic review of the current evidence has not been undertaken.

With aged horses making up a significant proportion of the equine population (Mellor et al., 1999; Ireland et al., 2011a), PPID is being diagnosed with increasing frequency (Rohrbach et al., 2012). Further to this, engagement with veterinary surgeons and owners with experience of PPID identified that evaluation of diagnostic test accuracy and factors which may affect it should be high on the research agenda (Chapter 2). It is therefore important to further investigate the accuracy of basal ACTH as the most commonly used diagnostic test. The aim of this systematic review was to collate and evaluate the current evidence regarding the sensitivity and specificity of the basal ACTH diagnostic test for the diagnosis of PPID.

Materials and methods

Search methodology

Based upon guidelines for electronic search strategies (McGowan et al., 2016), a systematic search of the veterinary literature was undertaken in January 2019, repeated in September 2020 and updated in January 2021. The following electronic databases were searched to ensure appropriate coverage (Grindlay et al., 2012); NCBI PubMed, Clarivate Analytics Web of Science, CAB Direct, SciVerse Scopus, and the database of the International Veterinary Information Service (IVIS). The bibliographies of retrieved publications were also screened for additional relevant records. Searches were conducted using a range of free text search terms and MEDLINE MeSH terms, including (Equine Cushing's syndrome), (pituitary pars intermedia dysfunction) and (Adrenocorticotrophic hormone [ACTH]). The full list of search terms and Boolean operators is detailed in Appendix 1. This review is specific to PPID in horses and ponies, therefore search terms relating to donkeys and other equids were not included. All retrieved records were imported and formatted into a pre-designed Microsoft Excel spreadsheet.

Inclusion criteria

Publication limitations on study design, publication date, setting or study population were not imposed. Although not peer-reviewed, conference proceedings were also considered for inclusion, but other grey literature sources were not. Only English language studies were included. However, English language abstracts were assessed, where available, for foreign language studies. Initial title screening was undertaken for all identified studies, and at this stage, textbook chapters, letters, review articles, non-equine studies and those not pertaining to the diagnosis of PPID were excluded. The abstracts of remaining studies were

subsequently screened and those that reported on other basal or dynamic diagnostic tests or did not contain data relevant to the diagnostic accuracy of basal ACTH for the diagnosis of PPID were excluded. All studies that reported, or contained sufficient data to calculate, sensitivity and/or specificity of the basal ACTH diagnostic test were included. If abstracts reported findings from the same study as a full publication, to avoid duplication only the full publication was assessed. Eligibility assessment was carried out independently in a standardised, unblinded manner by the authors.

Review methodology

Standardised forms adapted from critically appraised topic (CAT) data collection forms were developed *a priori* and used to extract relevant data from included publications. Data extraction and appraisal of included studies were undertaken individually by the doctoral student and subsequently cross-checked by the other authors to ensure a consensus was reached. No attempt was made to obtain missing data and investigators were not contacted to confirm accuracy of included information. Data and relevant results extracted from each included publication are presented in summary tables (Appendix 2).

Formal quality assessment scoring was not undertaken and studies were not excluded on quality grounds. However, results from lower quality studies are presented with interpretation of their limitations. For each included study, risk of bias and applicability concerns were appraised using the quality assessment tool for diagnostic accuracy studies (QUADAS-2; Whiting et al., 2011; Appendix 3). Due to the variations between included studies with respect to inclusion criteria and participant selection, which may alter the spectrum of disease and non-disease in the population, the use of non-comparable diagnostic assays and the risk of bias highlighted when studies were evaluated using the QUADAS-2 checklist, meta-analysis was not undertaken (Campbell et al., 2020). Performing meta-analysis on studies with important differences and a high risk of bias may compound the errors and produce an erroneous result which could be inappropriately interpreted as having credibility (Macaskill et al., 2010). However, median sensitivity and specificity estimates for basal ACTH were calculated, and where 95% confidence intervals (CI) were not provided in the publication, these were calculated by the authors where published information permitted. Mann-Whitney U tests were used to test the statistical significance of differences in median values of sensitivity and specificity between different ACTH assays and reference standards. Aspects of study design, methodology and information reported in

included studies are presented aligned with selected items included in the STARD (Bossuyt et al., 2015; Cohen et al., 2016), and STARD for abstracts (Cohen et al., 2017) checklists.

Results

A total of 795 records were identified via the combined electronic database searches, with an additional four records obtained from other sources. Following removal of duplicate records, 415 records were screened for relevance to the review question. Following title and abstract screening, a total of 25 publications were evaluated in full. Fourteen publications did not meet inclusion criteria and were subsequently excluded, resulting in 11 studies included in the review (Figure 1).

Study design and populations

Only five publications included reference to diagnosis of PPID within the title (van der Kolk et al., 1995; Horowitz et al., 2003; McGowan et al., 2013b; Rendle et al., 2015a; Horn et al., 2020), while two studies did not refer to any measure of diagnostic test accuracy within the abstract (Sojka et al., 2006; Beech et al., 2011b). None of the included studies completely fulfilled reporting guidelines for items to be included within abstracts for diagnostic accuracy studies (Cohen et al., 2017), primarily due to insufficient details regarding eligibility criteria and sample selection, precluding assessment of risk of bias.

While some publications utilised other designs for components of the study, for evaluating basal ACTH for the diagnosis of PPID, nine included publications represented case control selection cross-sectional studies (Mathes and Pieper, 2019), with a two-gate design using healthy controls (van der Kolk et al., 1995; Couëtil et al., 1996; Horowitz et al., 2003; Sojka et al., 2006; Beech et al 2011b; Rendle et al., 2015a), or a three-gate design, using healthy controls combined with suspected PPID cases (Perkins et al., 2002), or using healthy controls compared with separate groups of PPID and suspected PPID cases (Beech et al., 2007, 2011a). One further study enrolled a cohort of horses that were followed over a 12 month period, using case control selection with a two-gate design using healthy controls, and basal ACTH diagnostic accuracy was evaluated monthly in repeated cross-sectional studies (Horn et al., 2020). The remaining study used a cohort selection cross-sectional design with a single gate design (McGowan et al., 2013b).

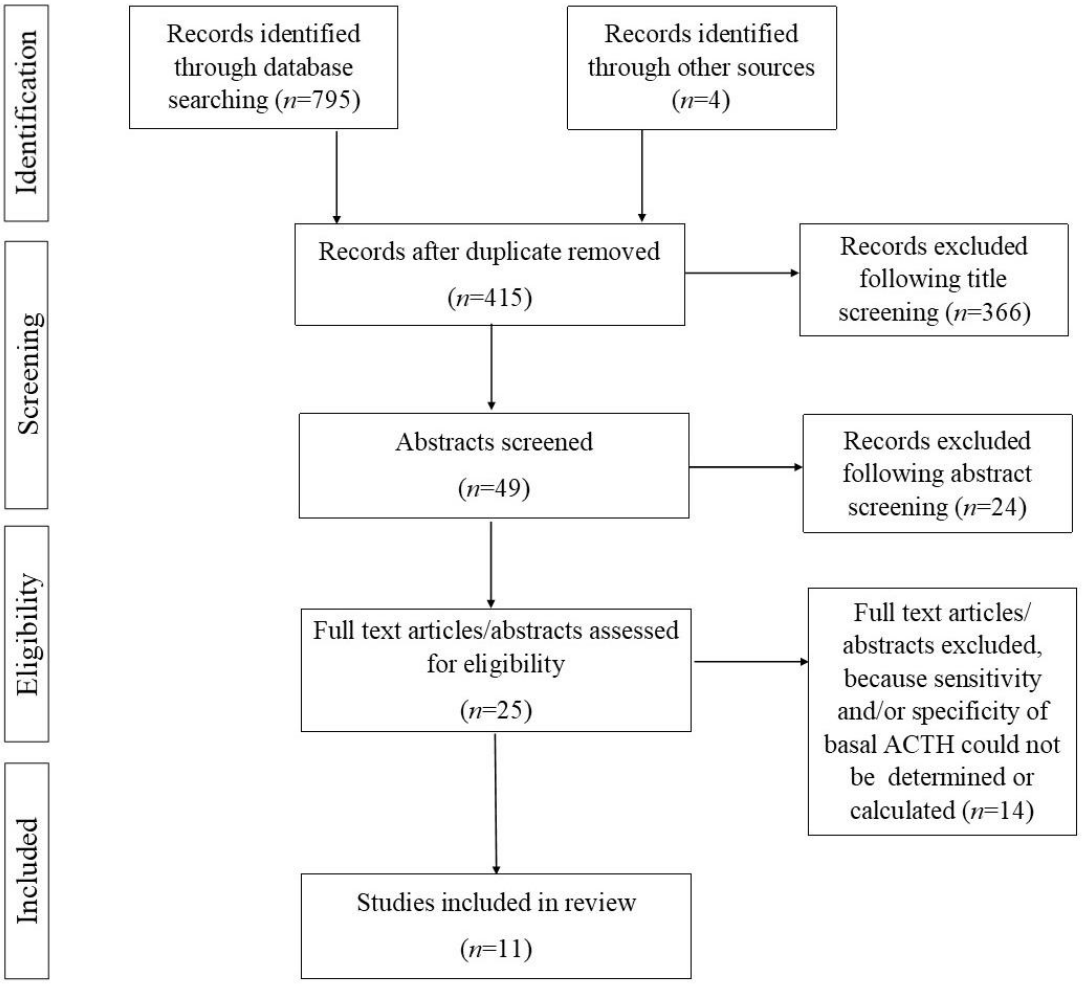


Figure 1: Flow diagram of the search strategy undertaken in a systematic review to identify and screen publications with information on the sensitivity and specificity of the basal plasma ACTH diagnostic test for identifying PPID in horses, resulting in the identification of 10 included publications (adapted from Moher et al., 2009).

No included studies enrolled a consecutive or random sample of horses/ponies presenting due to suspected PPID, and all included studies provided relatively limited description of how potentially eligible cases were identified and screened for inclusion. No studies reported information regarding previous test results as a component of their eligibility criteria, and only one study provided full details regarding the study location and the date range during which the study population was selected (Perkins et al., 2002). Four of the studies included referral hospital populations (van der Kolk et al., 1995; Couëtil et al., 1996; Perkins et al., 2002; Rendle et al., 2015a), and one used a field-based population (McGowan et al., 2013b). The remainder did not include sufficient information to determine study setting

(n=6), although it is probable that at least a proportion of their study populations were selected from referral hospitals (Table 1 and Appendix 2). Three studies reported that PPID cases were identified via hospital cases or clinical records, but provided limited information about the selection of controls (van der Kolk et al., 1995; Couëtil et al., 1996; Perkins et al., 2002). Regardless of the choice of reference standard, the most common method for selecting PPID cases for inclusion was a presumptive diagnosis based on clinical signs (n=6), with half of these studies including hypertrichosis as an inclusion criterion in all cases (van der Kolk et al., 1995; Couëtil et al., 1996; Perkins et al., 2002).

No studies stated an intended sample size, although one study aimed to sample at least 50 horses (including both cases and controls) per month during the study period (Horn et al., 2020), and all studies had small sample size of PPID cases (n<55), with the majority also having a small number of controls (n<50) (Table 1). Furthermore, in several studies, sample size was further reduced by performing subgroup analyses (van der Kolk et al., 1995; Couëtil et al., 1996; Beech et al., 2007, 2011a; McGowan et al., 2013b; Horn et al., 2020).

Inclusion criteria for controls also varied between studies. The majority of studies defined controls by the absence of clinical signs. However, in three studies (with some overlap between study populations), a subset of animals selected as controls on the basis of absence of clinical signs of PPID subsequently had post mortem examination where 46.5-58.3% had histological evidence of pars intermedia hyperplasia or adenoma (Beech et al., 2007, 2011a,b). Another study selected controls on the basis of absence of clinical signs of PPID and a maximum of two ACTH results that were identified as outlying values during a 12 month period, together with an absence of pituitary adenoma in a subset that had post mortem examination (Horn et al., 2020). One further study selected controls based on results of an ODST (Horowitz et al., 2003).

Only two studies used age-matched controls (Horowitz et al., 2003; McGowan et al., 2013b). One study only provided age data overall, rather than by group (Rendle et al., 2015a) and for all other studies, while mean or median age of the control group was younger compared to the PPID groups, the control group included at least one animal aged ≥15 years. Descriptions of baseline demographic characteristics of the study population were limited in all included studies. Where sufficient information was provided regarding the demographic composition of the control group, there were breed differences, particularly with respect to pony breeds being represented among PPID cases, but not the control group (van der Kolk

et al., 1995; Beech et al., 2007; 2011a,b), while one study's eligibility criteria specifically excluded ponies (Horn et al., 2020). There were also differences in sex distribution with a greater proportion of mares in the PPID group compared to controls (Beech et al., 2007; 2011a,b). Only one study provided details regarding clinical signs exhibited by PPID cases beyond the description of case inclusion criteria (Couëtil et al., 1996), meaning that the distribution of disease severity in PPID cases could not be ascertained in other included studies. The prevalence of clinical signs was reported for the entire study population in one publication, but not for the PPID and control groups used for assessment of diagnostic test accuracy (McGowan et al., 2013b).

Test methods: index test

Only three studies solely evaluated ACTH (Couëtil et al., 1996; Perkins et al., 2002; Rendle et al., 2015a) with all other studies including assessment of at least one other basal and/or dynamic test for PPID diagnosis. Basal ACTH was the index test (or one of the index tests) under evaluation in seven included studies. Of these, six stated the intended use of ACTH to be for diagnosis of PPID (van der Kolk et al., 1995; Couëtil et al., 1996; Horowitz et al., 2003; McGowan et al., 2013b; Rendle et al., 2015a; Horn et al., 2020) and one reported the intended use to be monitoring response to treatment (Perkins et al., 2002), with no publications defining the intended clinical role of basal ACTH. Other included studies were designed to evaluate various dynamic diagnostic tests for PPID, but reported data pertaining to basal ACTH and its diagnostic accuracy (Sojka et al., 2006; Beech et al., 2007; Beech et al., 2011a,b).

The diagnostic (positivity) cut-off value for ACTH used to identify PPID also varied between studies (Table 1). Five studies determined the ACTH diagnostic cut-off value *a priori* based on laboratory reference intervals (Perkins et al., 2002; Sojka et al., 2006; Beech et al., 2007; Beech et al., 2011a,b). Five studies derived single (van der Kolk et al., 1995; Horowitz et al., 2003), breed-adjusted (separate values for horses and ponies; Couëtil et al., 1996) or seasonal (separate values for autumn and non-autumn; McGowan et al., 2013b) or monthly (Horn et al., 2020) cut-off values as part of the study. In the latter study, monthly reference intervals based on basal ACTH concentrations for 40-50 control horses were also calculated (Horn et al., 2020). The final study derived different diagnostic cut-off values for both single and paired ACTH measurements (from two samples collected 5-15 minutes apart) (Rendle et al., 2015a). This study also presented agreement between ACTH and histopathology, which was assumed to utilise seasonally adjusted cut-off values (>47 pg/ml for autumn (August-

October) and >29 pg/ml for non-autumn (November–July); however, the cut-off values used for these analyses were not stated (Rendle et al., 2015a).

Pre-analytical factors such as sample handling varied between studies. Only five studies reported obtaining blood samples where horses were in familiar surroundings or kept quiet in stalls (Couëtil et al., 1996; Beech et al., 2007; 2011b; McGowan et al., 2013b; Horn et al., 2020), and only one study reported the diet offered around the time of sampling (Couëtil et al., 1996), with one further publication reporting that ad libitum food was available prior to sampling, although diet varied between included horses (Horn et al., 2020). Three studies reported use of glass ethylenediaminetetraacetic acid (EDTA) tubes for sample collection (Couëtil et al., 1996; Beech et al., 2011a; McGowan et al., 2013b), while three reported using plastic EDTA tubes (Beech et al., 2007; 2011b; Horn et al., 2020) and the remaining five studies did not report the type of tube used. Samples were centrifuged to separate plasma at time points ranging from immediately after collection to within 2 hours afterwards (Appendix 2). Plasma samples were frequently frozen prior to analysis, at between -20°C to -80°C, for periods of between 1 week and 2 years (Appendix 2). Additionally, included studies used two different diagnostic assays to determine ACTH concentrations; the chemiluminescent immunoassay (CIA; n=8) and the radioimmunoassay (RIA; n=3) (Table 1).

Test methods: reference standards

The reference standards utilised to confirm presence of PPID included histological evidence of pituitary adenoma (n=2; van der Kolk 1995; Sojka et al., 2006), pituitary hyperplasia or adenoma (n=3; Beech et al., 2011a,b; Rendle et al., 2015a), or pituitary hyperplasia (n=1; Beech et al., 2007); clinical signs of PPID (including hypertrichosis) (n=3; Couëtil et al., 1996; Perkins et al., 2002; McGowan et al., 2013b), and overnight dexamethasone suppression test (ODST; n=1; Horowitz et al., 2003) (Table 1). One study utilised the eligibility criteria used for identifying PPID cases as the reference standard, where horses had to meet at least two of three criteria, including; exhibiting clinical signs consistent with PPID, post mortem examination findings that confirmed or excluded pituitary adenoma or hyperplasia, or having ≥6 of the monthly ACTH results obtained that were statistical outliers within the 12 month study (Horn et al., 2020). Histopathological grade was not reported in any study using pituitary hyperplasia for the reference standard.

No studies included a flowchart or other graphical representation of flow and timing of recruitment, index and reference standard tests. Studies utilising histopathology as the reference standard, necessarily conducted all index tests (ACTH measurements) prior to the reference test, although only one of these studies provided information regarding the time interval between ACTH measurement and post mortem examination (Beech et al., 2007). The study utilising the ODST as the reference standard did not report the time interval between this and ACTH measurement (Horowitz et al., 2003). For studies using clinical sign(s) as the reference test, the time between observation of clinical signs and measurement of basal ACTH was not reported, with the exception of one study, where ACTH measurement was performed at the time of veterinary examination, although the reference standard comprised a combination of examination findings and owner-reported clinical signs observed within the preceding 12 months (McGowan et al., 2013b).

Sensitivity and specificity of basal ACTH measurement for the diagnosis of PPID

Only five studies included all enrolled cases and controls in the evaluation of the diagnostic accuracy of basal ACTH (Couëtil et al., 1996; Perkins et al., 2002; Horowitz et al., 2003; Sojka et al., 2006; Beech et al., 2011b). Overall in all included papers there were a total of 234 cases and 558 controls that contributed data towards analysis of diagnostic accuracy. The median sensitivity was 75.5% (IQR 64.0-86.5%; range 36.0-100%; based on 22 estimates provided in all 11 included studies) and median specificity was 95.2% (IQR 84.2-98.9%; range 63.3%-100%; based on 21 estimates provided in ten included studies) (Table 1). The lowest sensitivity reported across all studies was calculated where a diagnostic cut-off of >35 pg/ml was applied for the diagnosis of histological evidence of pituitary hyperplasia in clinically normal horses exhibiting no signs of PPID (Beech et al., 2007; Table 1).

When studies were grouped by the type of assay used, reported sensitivity was significantly higher in studies using the RIA assay ($p=0.04$), whereas reported specificity did not differ significantly between assay types ($p=0.19$). For those using the CIA assay the median sensitivity was 71.0% (IQR 60.6-84.4%; range 36.0-100%) and median specificity was 94.6% (IQR 82.1-96.5%; range 63.3-100%), while those using the RIA assay had a median sensitivity of 87.5% (IQR 82.4-97.7%; range 81.8-100%) and a median specificity of 100% (IQR 89.5-100%; range 89.5-100%). When studies were grouped by reference standard (excluding the single study using ODST (Horowitz et al., 2003) and the study where the composite reference standard varied between horses (Horn et al., 2020), reported sensitivity

was significantly higher in studies using a clinical reference standard (p=0.04), whereas reported specificity did not differ significantly between reference standards (p=0.71). For studies that used histology as the reference standard, the median sensitivity was 65.0% (IQR 56.8-71.0%; range 36.0-100%) and the specificity was 95.6% (IQR 86.0-100%; range 80.9-100%), while for those using clinical signs of PPID, the median sensitivity was 82.9% (IQR 74.8-93.2%; range 59.0-100%) and the median specificity was 94.6% (IQR 81.4-100%; range 78.0-100%).

Table 1: Summary of sensitivity and specificity of the basal plasma adrenocorticotrophic hormone concentration for diagnosing pituitary pars intermedia dysfunction (PPID) in horses.

Study	Type of ACTH assay	Reference standard	Number of cases included in analysis	Number of controls included in analysis	Age-matched controls	Country and Setting	Sensitivity (95% confidence intervals (CI) included where reported or calculated where data allowed)	Specificity (95% confidence intervals (CI) included where reported or calculated where data allowed)	Diagnostic (positivity) cut-off point of ACTH for PPID
Beech et al., 2007[#]	CIA	Histological PH on PM	6	10 (clinically normal with no evidence of PH)	No	USA Not stated	71.0%	100%	>35 pg/ml
		Clinically normal but PH on PM	14	10 (clinically normal with no evidence of PH)			36.0%	100%	
Beech et al., 2011a[#]	CIA	Histological PH or PA on PM (in horses with ≥2 clinical signs of PPID)	25	23 (clinically normal with no evidence of PH)	No	USA Not stated	71.0%	96.0%	≥36 pg/ml
		Histological PH or PA on PM (in horses with ≥2 clinical signs of PPID, plus horses with ≥1 clinical sign of PPID which did not include hair coat abnormalities)	35	23 (clinically normal with no evidence of PH)			65.0%	96.0%	

		Histological PH or PA on PM (in horses with ≥2 clinical signs of PPID)	25	53 (all clinically normal animals including those with no PM examination, or no evidence of PH, or with histological PH on PM)			71.0%	86.0%	
		Histological PH or PA on PM (in horses with ≥2 clinical signs of PPID, plus horses with ≥1 clinical sign of PPID which did not include hair coat abnormalities)	35	53 (all clinically normal animals including those with no PM examination, or no evidence of PH, or with histological PH on PM)			65.0%	86.0%	
Beech et al., 2011b	CIA	≥2 clinical signs of PPID (n=10) or equivocal clinical signs (n=3) (of these, n=8 had confirmed PH or PA on PM)	13	19 (all clinically normal animals, n=14 had PM of which n=7 had evidence of PH)	No	USA Not stated	59.0% (CI 32.9-81.6%)	94.0% (CI 74.0-99.9%)	>36 pg/ml
Couëtil et al., 1996	RIA	Hypertrichosis (defined as excessive long hair growth, delayed or incomplete shedding) plus ≥1 additional clinical sign (n=18) histological PA on PM (n=4)	11 horses	18 horses (clinically normal)	No	USA University referral hospital	90.9% horses	100% horses	Horses >50 pg/ml
			11 ponies	9 ponies (clinically normal)			81.8% ponies	100% ponies	Ponies >26.96 pg/ml

Horn et al., 2020#	CIA	2 out of 3 of: i) clinical signs of PPID (hypertrichosis or delayed shedding and epaxial muscle wastage, abnormal fat distribution or abnormal sweating); ii) PM findings confirming or excluding PA or PH; iii) outlying basal or post-TRH ACTH values for ≥6 tests (out of maximum 12 monthly tests)	7	44 (clinically normal; unreported number did not have PA on PM)	No	Australia Not stated	100% (CI 64.6-100%)	95.4% (CI 84.5-99.2%)	April (autumn) >79.1 pg/ml
			10	41 (clinically normal; unreported number did not have PA on PM)			80.0% (CI 49.0-96.5%)	72.5% (CI 57.2-83.9%)	November (spring) >25.7 pg/ml
			9	46 (clinically normal; unreported number did not have PA on PM)			88.9% (CI 56.5-99.4%)	97.8% (CI 88.7-99.9%)	May (autumn) >41.6 pg/ml
			7	50 (clinically normal; unreported number did not have PA on PM)			85.7% (CI 48.7-99.3%)	63.3% (CI 49.3-75.3%)	March (autumn) >58.3 pg/ml
Horowitz et al., 2003	RIA	ODST	25	38 (negative ODST result)	Yes	Not stated	84.0%	89.5%	>11pmol/l (>50 pg/ml)
McGowan et al., 2013b	CIA	Owner-reported hypertrichosis (defined as excessive long hair growth, and/or delayed shedding and/or failure to shed coat normally) plus ≥3 additional clinical signs	20	217 (no clinical signs of PPID)	Yes	Australia Field-based	Non-autumn: 80.0% (CI: 56.3-94.3%)	Non-autumn: 82.5% (CI: 76.8-87.3%)	Non-autumn >29.7 pg/ml
			4	63 (no clinical signs of PPID)			Autumn: 100% (CI: 39.8-100.0%)	Autumn: 95.2% (CI: 86.7-99.0%)	Autumn >77.4pg/ml
Perkins et al., 2002	CIA	Hypertrichosis (defined as hirsutism)	19	49 (healthy controls combined with possible PPID cases; hypertrichosis not present)	No	USA University referral hospital	84.0% (CI: 60.0-97.0%)	78.0% (CI: 63.0-88.0%)	>35 pg/ml

Rendle et al., 2015a	CIA	Histological PH or PA on PM	44	23 (normal PI on PM)	No	Australia, USA and UK Two university referral hospitals, one private hospital and research herds	69.4%	80.9%	>21.3 pg/ml
							61.1%	95.2%	>23.6 pg/ml
							56.8% (CI: 41.0-71.7%)	*91.3% (CI: 72.0-98.9%)*	Not stated but presumed to be based on reference intervals reported in methods: Non-autumn >29 pg/ml Autumn >47 pg/ml
Sojka et al., 2006	CIA	Histological PA on PM	5	2	No	Not reported	40.0% (CI: 5.3-85.3%)	*100% (CI: 15.8-100.0%)*	>59 pg/ml
van der Kolk et al., 1995	RIA	Histological PA on PM	16	7 (clinically healthy animals)	No	The Netherlands University referral hospital	100%	N/R	>55 pg/ml
Overall			[#]Total number of cases n=234	[#]Total number of controls n=558			**Median sensitivity** *75.5% (IQR 64.0-86.5%; range 36.0-100%)*	**Median specificity** *95.2% (IQR 84.2-98.9%; range 63.3-100%)*	

PH= pituitary hyperplasia; PA= pituitary adenoma; ACTH= adrenocorticotrophic hormone; RIA=radioimmunoassay; ODST= overnight dexamethasone suppression test; CIA = Chemiluminescent immunoassay; PM = post mortem; N/R Not reported or insufficient data available; *Sensitivity and/or specificity calculated from data provided

[#]Subgroup analyses presented in separate rows: for Beech et al., 2007, maximum sample size included in diagnostic accuracy data analysis was n=20 PPID cases (6 confirmed cases plus 14 clinically normal animals with histological evidence of PH) and n=10 controls (clinically

normal horses without histological evidence of PH); for Beech et al., 2011a, maximum sample size included in diagnostic accuracy data analysis was n=35 PPID cases (suspected and confirmed cases) and n=53 controls (clinically normal horses with or without histological evidence of PH, and those without PM examination); for Horn et al., 2020, only the data from months with the highest and lowest reported values for sensitivity (identical sensitivity reported for both November and December, with November included in table due to slightly larger sample size) and specificity are presented, and maximum sample size included in diagnostic accuracy data analysis was n=11 PPID cases (sampled during July) and n=50 controls (sampled during March). Only these maximum sample sizes are included in the total numbers of cases and controls.

Discussion

This is the first study to systematically review published evidence regarding the sensitivity and specificity of measurement of basal ACTH for the diagnosis of PPID in horses and ponies. Overall, the basal ACTH test was reported to have good sensitivity and excellent specificity in the majority of studies with overall medians of 75.5% and 95.2%, respectively. However, sensitivity varied more widely among studies with it reported to be <60% in two studies, which both used ungraded histopathology as the reference standard (Sojka et al., 2006; Beech et al., 2011b).

QUADAS-2 and STARD assessment highlighted that all included studies were at risk of significant biases, which can lead to inappropriate recommendations about testing protocols (Whiting et al., 2011; Cohen et al., 2016, 2017). In many cases, studies provided limited information regarding eligibility criteria and sample selection meaning full assessment of related biases was not possible. It was therefore considered that combination of numerical data was not appropriate and meta-analysis was not undertaken. Meta-analysis of biased results can result in misleading results and is likely to compound errors, producing an erroneous result that is incorrectly interpreted as having credibility (Macaskill et al., 2010). Additionally, the studies included here varied considerably in their design, using different reference standards, assays and diagnostic cut-off values. In instances such as this, meta-analysis has been described a 'combining apples with oranges' (Macaskill et al., 2010) and analysis of such varied studies would produce a meaningless result or obscure differences in measures of diagnostic accuracy where clinical and/or methodological characteristics vary between studies (Macaskill et al., 2010). Therefore, undertaking meta-analysis would likely have led to inappropriate recommendations and could ultimately negatively affect case outcomes. However, effective data synthesis can still be undertaken without meta-analysis by following synthesis of results guidelines (McInnes et al., 2018; Salameh et al., 2020), evaluation check lists and calculating summary statistics (Campbell et al., 2020) as presented here.

Overall, none of the studies identified utilised optimal study designs for the assessment of diagnostic tests. Ideally a diagnostic test should be assessed using a prospective cohort of patients representative of the population to which it will be applied (Drobatz, 2009; Linnet et al., 2012), however none of the included studies enrolled a consecutive or random sample of horses/ponies presenting due to suspected PPID. How the study population is selected greatly influences the results of the diagnostic test being assessed and how applicable any

findings are to the target population (Vetter et al., 2018). Although no included studies enrolled a cohort of horses suspected of having PPID, the field-based study by McGowan et al., (2013b) enrolled a population of horses/ponies aged ≥15 years. Age is a risk factor for PPID (Ireland and McGowan, 2018), which is predominantly diagnosed in horses aged ≥15 years (Welsh et al., 2016). Therefore, the age range of included animals would define them as at risk of PPID (McGowan et al., 2013b), providing an indication to perform a diagnostic test (Mathes and Pieper, 2019). The other ten included studies utilised a case control design to select the study population, enrolling suspected cases of PPID based on presentation of clinical signs and a healthy control group defined by a lack of clinical signs. This method of using highly selected animals is likely to increase the risk of various biases that exaggerate diagnostic accuracy (Whiting et al., 2004, 2013; Hall et al., 2019) and to increase the likelihood of reporting clinically unrepresentative estimates (Linnet et al., 2012).

PPID is a progressive disease and therefore, like many diseases, has a spectrum of clinical signs that should be represented in the sample population in which the test characteristics were developed (Drobatz, 2009). The sensitivity and specificity of a test varies with the prevalence or distribution of a disease in the population or sample (Irwig et al., 2002; Leeflang et al., 2009). Therefore, when studies include a narrow range of individuals, such as described here, spectrum bias is observed and both sensitivity and specificity are falsely increased (Usher-Smith et al., 2016; Hall et al., 2019; Kea et al., 2019). One study in particular excluded horses based on some clinical signs, effectively selecting the 'sickest and wellest' animals (McGowan et al., 2013b) meaning a small spectrum of more advanced disease was represented. The fact that the median sensitivity for studies using clinical signs as the reference standard was greater than the overall median sensitivity, and significantly higher than when histology was used, supports the potential for exaggerated diagnostic accuracy due to spectrum bias. Additionally, none of the included studies reported a predefined sample size that they attempted to reach and enrolled small number of cases and controls, resulting in sample sizes below those recommended for evaluating sensitivity and specificity (Bujang and Adnan, 2016). This may be due in part to ethical considerations, such as performing unnecessary invasive procedures in healthy controls or clearly diseased animals.

All included studies met the definition of a diagnostic accuracy study, and were therefore described as cross-sectional studies (Mathes and Pieper, 2019). However, only one study that utilised histopathology as the reference standard and provided sufficiently detailed

information to accurately determine the time interval between the conduct of index and reference tests (Beech et al., 2007). If a substantial time period elapsed between ACTH measurement and subsequent post mortem examination in other studies using histopathology, these studies may more correctly be described as longitudinal studies and may reflect predictive accuracy of elevated ACTH for the future development of pars intermedia hyperplasia or adenoma (Mathes and Pieper, 2019). Since PPID is largely considered to be slowly progressive in the majority of cases, the likelihood of delayed verification bias may be small.

Five of the included studies used pituitary pars intermedia histopathology as the reference standard. Descriptions of pituitary histological findings in PPID demonstrate a range of pathology from hyperplasia and enlargement of the pars intermedia, through to micro and macroadenomatous changes (Boujon et al., 1993; Yoshikawa et al., 2001). However, poor agreement between pathologists has been reported, with some participating pathologists reporting normal histologic findings in eight out of ten suspected PPID cases where other pathologists reported pars intermedia hyperplasia and/or adenomatous hyperplasia or adenoma (McFarlane et al., 2005a). Other reports have indicated discrepancies between clinical findings and histopathology, with pars intermedia lesions evident in up to 46% of apparently clinical normal horses (van der Kolk et al., 2004; Miller et al., 2008); with 28% of geldings, 55% of lactating and 84% of pregnant mares showing pars intermedia hyperplasia, microadenoma or macroadenoma (van der Kolk et al., 2004). Pituitary glands, obtained at post mortem examination from clinically normal horses, had significantly higher histologic grades, with 12 of 34 (35%) glands classified as grade 3 (pars intermedia adenomatous hyperplasia) and 7 of 34 (21%) as grade 4 (pars intermedia microadenomas) during the autumn months, compared to 27% and 5%, respectively, in non-autumn months (Cordero et al., 2012). The same study also reported that pituitary size and pars intermedia area were significantly higher in the autumn compared to non-autumn months (Cordero et al., 2012). These studies indicate that pituitary intermedia histopathology is influenced by both reproductive status and season, and may have poor specificity for the diagnosis of PPID. Collectively, these findings do not support pituitary histopathology as the gold standard for diagnosis of diagnosis of PPID.

Four studies used clinical signs of PPID including (including hypertrichosis) as the reference standard for some (Couëtil et al., 1996; Beech et al., 2011b) or all PPID cases (Perkins et al., 2002; McGowan et al., 2013b). Hypertrichosis has been reported to be a specific

indicator of PPID (Schott, 2002), with a significant difference between PPID cases and controls when hypertrichosis was graded on a scale of 0-3 (Schott et al., 2017) and was reported to have high specificity (95%) for the diagnosis of PPID in one study (Frank et al., 2006a) when compared to histological evidence of pars intermedia adenoma. However, overt generalised hypertrichosis is now considered an advanced clinical sign of PPID (EEG, 2019), and therefore is unlikely to be representative of the earlier stages of disease. Only one study used owner-reported delayed shedding and hair coat changes over the previous 12 months as well as overt hypertrichosis identified on clinical examination (McGowan et al., 2013b). Compared to veterinary clinical examination findings, owner observations over a longer time period are likely to be more sensitive for detecting regional hypertrichosis and other subtle hair coat changes, and owner-reported history of hypertrichosis was the only clinical sign found to be predictive for PPID in a large cross-section of geriatric horses (McGowan et al., 2013a). One study used the ODST as the reference standard (Horowitz et al., 2003). The ODST was once thought of as the 'gold standard' diagnostic test for PPID reportedly having 100% sensitivity and specificity of 100% (Dybdal et al., 1994). However, this has subsequently been brought into question by a small study that reported only one of seven PPID cases had positive ODST results when repeated on three occasions at monthly intervals (Miesner et al., 2003) and concerns around the unnecessary administration of steroid means it is now seldom used in the UK. Given the lack of a true gold standard reference test for the diagnosis of PPID, future evaluation of diagnostic test accuracy could be improved by utilising methods to assess diagnostic test accuracy in the absence of an ideal reference standard (Umemneku Cikere et al., 2019), a composite reference standard (Naaktgeboren et al., 2013), or expert panel diagnosis (Bertens et al., 2013).

ACTH is not the major pituitary peptide normally produced from the equine pituitary pars intermedia, with its typical site of production and regulation being the pars distalis. However, it was shown to be increased (along with other POMC-derived peptides) in high amounts from diseased pars intermedia, and much of that was shown to be biologically inactive, which is why it remains a useful diagnostic test for PPID (Orth et al., 1982; Okada et al., 1997; Cordero et al., 2011). However, pars distalis produced ACTH as part of the hypothalamic-adreno-cortical axis may still provide false elevations of ACTH. ACTH levels have been shown to elevate as a result of factors such as stress (Fazio et al., 2008), exercise (Nagata et al., 1999), diet (Diez de Castro et al., 2014; Jacob et al., 2017), discomfort (Gehlen et al., 2020) and hospitalisation, even in mildly ill horses (Towns et al, 2010). These changes are often short lived but are still an important consideration when

interpreting ACTH concentration. There was a lack of information regarding the management of horses/ponies around the time samples were collected or where and how samples were taken, with six studies providing no information at all. If sampling took place in an environment that was not properly controlled to prevent the influence of external factors, ACTH levels may have been inadvertently affected, resulting in biased results. However, two of the studies also measured α-MSH, which is not affected by external influences like ACTH, and reported broadly comparable measures of diagnostic accuracy (Horowitz et al., 2003; McGowan et al., 2013b), and a strong positive correlation between α-MSH and ACTH (McGowan et al., 2013b). The description of demographic characteristics, and presence of differential or alternative diagnosis or comorbidities amongst study populations included in most reviewed publications was also poor, meaning the assessment of applicability to the target population was not able to be assessed fully (Drobatz, 2009; Linnet et al., 2012). However, the high proportion of hospitalised populations suggest the results may not be entirely generalisable in practice, and reported measures of diagnostic accuracy might be affected by referral filter bias.

Other pre-analytical factors also varied between studies. Once samples had been collected, there was variability in sample handling, which can affect ACTH concentrations (Hegstad et al., 1990; Teahan et al., 2006; Hirayama et al., 2015; Rendle et al., 2015c). Additionally, one study (McGowan et al., 2013b) stored samples for a prolonged period of time, meaning samples may have degraded by the time of analysis, potentially affecting ACTH measures and producing inaccurate results (Banse et al., 2020).

The CIA assay was most commonly used among studies with three studies using the RIA assay. Two method comparison studies have shown significant differences in results using different assays when measuring ACTH levels in equids (Knowles et al., 2018; McGilvray et al., 2020). A cohort of 85 ponies had samples collected in the autumn and spring months with ACTH levels measured using both CIA and immunofluorescent (IF) assays, which yielded significantly different results in both seasons ($p<0.001$), with the IF assay results proportionally lower (Knowles et al., 2018). In healthy adult horses, ACTH values from both CIA and IF assays were positively correlated, but differed in both November and May with significantly higher values from the CIA (McGilvray et al., 2020). Here, the median sensitivity and specificity of the CIA assay was lower than the RIA assay, suggesting similar differences between these assays may be observed. However, this finding may also be subject to

confounding due to differences in reference standards, case selection and definition, or other variables.

No studies reported any blinding of observers with respect to test results, though this unlikely to be source of introduced bias for ACTH where the index test is an objective measure, compared to a diagnostic test that requires clinical interpretation (Whiting et al., 2011). The diagnostic cut-off values for ACTH varied between studies which can result in an increase or decrease in sensitivity and specificity, depending on the cut-offs used (Sharma and Jain, 2014). Moreover, selecting the diagnostic cut-off value to maximise test performance, as performed in half of the studies reviewed here, increases the risk of overestimating measures of diagnostic test accuracy (Cohen et al., 2016). Due to this between study variation, estimating overall sensitivity and specificity was challenging. A correlation between sensitivity and specificity can be calculated to assess heterogeneity in thresholds, however, this is unreliable where studies have small sample sizes (Moses et al., 1993; Leeflang et al., 2013) and was therefore not undertaken. The overall median sensitivity and specificity reported in this systematic review should simply be treated as a summary of reported results and interpreted with appropriate caution, based on the limitations of included studies. Seasonally adjusted reference intervals have been developed for ACTH in equids to account for the higher plasma ACTH observed in autumn months (Copas and Durham, 2012) and two included studies derived monthly (Horn et al., 2020) or seasonal cut-off values for autumn and non-autumn periods (McGowan et al., 2013b). However, subsequently an Australian review has recommended a third interval between the autumn and non-autumn cut-offs (Secombe et al., 2018), and more recently a large field-based study proposed weekly thresholds for ACTH to account for continuous circannual variation (Durham et al., 2020). Most studies selected the diagnostic cut-off value based on the upper limit of laboratory reference intervals (Perkins et al., 2002; Sojka et al., 2006; Beech et al., 2007, 2011a; 2011b) or derived single cut-off values (van der Kolk et al., 1995; Horowitz et al., 2003) and made no allowance for circannual variation, suggesting they may have been insufficient for accurate differentiation between disease and non-diseased animals.

Overall, the available evidence demonstrates that measurement of basal ACTH is highly specific and has good sensitivity for the diagnosis of PPID in horses and ponies exhibiting clinical signs consistent with the disease. However, the evidence base is limited, with selection of study populations and study designs used in the included studies likely to over-

estimate measures of diagnostic accuracy. Ideally, a cohort selection cross-sectional study, enrolling a consecutive sample of horses presenting due to suspicion of PPID, using a diagnostic cut-off value that is defined *a priori*, would be performed to appropriately evaluate the diagnostic accuracy of basal ACTH. However, it is essential that all horses would be correctly classified as diseased or non-diseased, and the lack of a gold standard reference test for PPID makes this particularly challenging.

Conclusion

None of the included studies utilised an optimal study design or study population for the evaluation of a diagnostic test and the majority were subject to bias. Furthermore, the amount of inter-study variations precluded direct comparison and meta-analysis. Despite this, basal ACTH was consistently reported to have good-excellent specificity, and while sensitivity estimates were more varied, overall medial sensitivity for the diagnosis of PPID was good in selected populations of horses and ponies. Basal ACTH performed better at ruling out PPID than detecting it, meaning a risk of missing some cases of PPID.

Declaration

This chapter was included with permission from the co-authors of the published version.

C. McGowan contributed to independent review of included studies and preparation and approval of the manuscript. J. Ireland contributed to independent review of included studies, study design and preparation and approval of the manuscript.

Appendices for Chapter 3

Appendix 1: Search strategy and results

Databases searched and dates covered	NCBI PubMed (1950 – Present) Clarivate Analytics Web of Science (1898 – Present) CAB Direct (1960 – Present) Scopus (1823 – Present) International Veterinary Information Service (IVIS) database (1997 – Present) Further relevant records were identified by the authors via the bibliographies and reference lists of retrieved publications and freely available or published conference proceedings.
Search terms	(Equine Cushing* OR Pituitary pars intermedia dysfunction OR PPID OR Hyperadrenocorticism OR Pituitary adenoma) AND (horse* OR pony OR ponies OR equine OR equid*) AND (ACTH OR adrenocorticotropic hormone OR adrenocorticotrophic hormone OR adrenocorticotropin OR corticotropin)
Dates searches performed	11/01/2019, 14/09/2020, 15/09/2020 and 04/01/2021

Database	Number of results	Excluded – non-English language publication	Excluded – conference abstract subsequently available as published article	Excluded – non-systematic review article, review in conference proceedings or letter	Excluded – not relevant to review question	Excluded – did not report data required to answer review question	Total relevant papers
NCBI PubMed	134	0	0	16	84	25	9
Clarivate Analytics Web of Science	145	1	0	21	89	25	9
CAB Direct	170	1	0	53	89	18	9
Scopus	174	0	0	56	90	19	9
International Veterinary Information Service (IVIS) database	172[#]	0	0	102	61	4	1
Other sources	4	0	1	0		3	0
Total relevant papers when duplicates removed, following title and abstract screening							**11**

#Row total does equal total number of results retrieved from IVIS searches, since the single relevant paper was included 5 times in total within the database search

Appendix 2: Summary tables and data of included studies

A summary of extracted data for each included study, main limitations of the studies and conclusions are presented in the tables below.

ACTH = Adrenocorticotrophic hormone

α-MSH = Alpha-Melanocyte-stimulating hormone

CIA = Chemiluminescence assay

CI = Confidence interval

EDTA = Ethylenediaminetetraacetic acid

ODST = Overnight dexamethasone suppression test

PPID = Pituitary pars intermedia dysfunction

RIA = Radioimmunoassay

ROC = Receiver operating characteristic

TRH = Thyrotropin-releasing hormone

Author, year: **Type of publication:**	Beech et al., 2007 Journal publication
Study Design:	Case control selection cross-sectional study; multiple-gate design using healthy controls
Aim/Objective of the Study:	To compare the effect of TRH administration on ACTH concentrations in healthy horses and those with PPID and to compare the TRH stimulation test with the ODST.
Setting:	Not stated. n=4 horses at university referral hospital in Canada; all others mid-Atlantic region of the United States (presumed to have been cases attending a university referral hospital).
Study Population:	No information provided regarding study population selection. n=48: Controls: n=29 clinically normal horses (breeds not reported, no ponies) • no clinical signs of PPID • n=16 aged ≤10 years; n=9 aged >10 - <15 years; n=4 aged >15 - 20 years • 20 geldings, 1 stallion and 8 mares

	• within this group, 1 horse developed clinical signs of PPID after the first TRH test and further tests were performed when the horse was clinically abnormal PPID cases: n=15 • 11 horses and 4 ponies with ≥2 clinical signs of PPID • n=1 aged ≤10 years; n=3 aged >10 - <15 years; n=3 aged >15 - 20 years; n=8 aged >20 years • 4 geldings and 11 mares Horses with equivocal signs of PPID: n=4 • appearance was suspicious but not classical or only had 1 sign suggestive of PPID; none had hypertrichosis • n=1 aged >10 - <15 years; n=1 aged >15 - 20 years; n=2 aged >20 years • 3 geldings and 1 mare Post mortem examination was undertaken within 22 days of a TRH stimulation test for n=24 animals without signs of PPID, n=4 animals with equivocal signs of PPID, and n=5 animals with signs of PPID. For one further PPID case, post mortem was undertaken 150 days after TRH test.
Diagnostic Test/Assay Investigated:	All except 4 TRH stimulation tests were performed by the same technicians, and horses were maintained in familiar surroundings. Animals were catheterised 30 minutes prior to basal sample collection, and 2 baseline samples were obtained 5 minutes apart. Blood samples were collected in plastic potassium EDTA tubes and centrifuged to separate plasma within 45 minutes of collection. Plasma was stored at -20°C in plastic tubes, then placed on ice packs, and sent by overnight post to the Animal Health Diagnostic Center at Cornell University for analysis. ACTH measured by immunometric chemiluminescence assay (CIA). Cut-off value of >35 pg/ml used for plasma ACTH concentration at baseline and both time points following TRH administration.
Outcome Measures:	Baseline ACTH concentrations in PPID and normal horses. Secondary outcome was to compare baseline ACTH in PPID and non-PPID horses to post mortem results.
Main Findings:	All 6 PPID cases and all 4 cases with equivocal clinical signs that had post mortem examination had histologic evidence of pars intermedia hyperplasia. Of the 24 clinically normal animals that had post mortem examinations, 14 had histologic evidence of pars intermedia hyperplasia. Baseline ACTH measured for 2 samples obtained 5 minutes apart varied minimally for clinically normal horses but were more variable in the PPID cases. Normal basal plasma ACTH concentrations (≤ 35 pg/ml): • Clinically normal horses: ▫ 6/7 tests from 5 controls that did not have post mortem examination ▫ 10/10 controls with no pituitary hyperplasia at post mortem

	▫ 9/14 controls with pituitary hyperplasia at post mortem • Equivocal clinical signs horses: ▫ 3/8 tests from 4 cases with pituitary hyperplasia at post mortem • PPID cases: ▫ 6/16 tests from 9 PPID cases that did not have post mortem examination ▫ 2 PPID cases with pituitary hyperplasia at post mortem Using histopathology as the reference standard, and a cut-off of >35 pg/ml, basal ACTH had a sensitivity of 71% and specificity of 100%, based on 10 clinically normal animals with no pituitary hyperplasia and 6 PPID cases with pituitary hyperplasia. In clinically normal animals, basal ACTH had a sensitivity of 36% and specificity of 100%, based on 10 controls with no pituitary hyperplasia and 14 controls with pituitary hyperplasia. For histological diagnosis of PPID (regardless of clinical signs, combining 6 PPID cases with pituitary hyperplasia and 14 clinically normal horses with pituitary hyperplasia) using an ACTH cut off of >35 pg/ml: sensitivity 100%, positive predictive value 72%, and diagnostic accuracy 72%. No animals without pituitary hyperplasia had basal ACTH >35pg/ml, therefore it was not possible to calculate specificity.
Interpretation of Results:	Small sample size and splitting to subgroups for analysis reduces study power further. Different subgroups used for classifying animals based solely on clinical presentation, and for classification based on both clinical presentation and pituitary histopathology. Likely that at least a proportion of the study population were from a referral hospital population but information on sample selection limited therefore unable to assess selection bias and ability to generalise to other equine populations. Using control population selected based on absence of clinical signs of PPID (i.e. not suspected PPID cases) may introduce bias to estimates of diagnostic accuracy. Study uses histopathology as reference standard for diagnosis of PPID. Baseline ACTH showed variability between samples collected 5 minutes apart, especially in PPID cases. Seasonal effect on ACTH observed, but study did not use seasonally adjusted reference intervals. All horses with histologically normal pituitary glands had low baseline ACTH concentrations (≤ 35 pg/ml), but normal basal ACTH concentrations were detected in some horses with histological evidence of PPID. Basal ACTH was shown to have good sensitivity and high specificity for diagnosis of PPID.

Author, year: **Type of publication:**	Beech et al., 2011a Journal publication

Study Design:	Case control selection cross-sectional study (for component of study evaluating measures of ACTH diagnostic test accuracy); multiple-gate design using healthy controls
Aim/Objective of the Study:	To compare ACTH and α-MSH after administration of TRH, and to compare ACTH concentrations after TRH administration with those following domperidone administration, in healthy horses and horses with PPID.
Setting:	Not stated
Study Population:	No information provided regarding study population selection. Substantial proportion of study population (n=48) also included in authors' previous study (Beech et al., 2007). n=88 Controls: n=69 clinically normal horses (mixed breeds, n=3 ponies) • no clinical signs of PPID • of these animals, ACTH was evaluated for: ▫ 10 animals with no post mortem ▪ 5 geldings, 1 stallion, 4 mares; mean age (± standard deviation) 8±2 years (range 4-11 years) ▫ 23 with no histologic pituitary changes ▪ 17 geldings, 6 mares; mean age 8±4 years (range 5-18 years) ▫ 20 with histologic pituitary hyperplasia or adenoma ▪ 11 geldings, 9 mares; mean age 16±7 years (range 5-29 years) PPID cases: n=47 • animals with ≥2 clinical signs of PPID or suspected to have PPID (based on ≥1 clinical sign, other than hypertrichosis or abnormal coat shedding, plus pituitary adenoma at post mortem) • post mortem examination performed on 34 PPID cases, all of which had histologic evidence of pituitary adenoma • of these animals, ACTH was evaluated for: ▫ 25 PPID cases ▪ 9 geldings, 16 mares; mean age 21±7 years (range 8-30 years); 4 of the 7 ponies/pony crosses in the study population were in the PPID group ▫ 10 suspected PPID cases ▪ 7 geldings, 3 mares; mean age 16±5 years (range 11-24 years)
Diagnostic Test/Assay Investigated:	Animals were catheterised 30 minutes prior to basal sample collection, and 2 baseline samples were obtained 5 minutes apart (except one horse with single baseline sample), and mean of the 2 samples was used as baseline value (except one horse with single sample). Blood samples were collected in glass EDTA tubes and centrifuged to separate plasma within 2 hours of collection. Plasma was stored at -70°C in plastic tubes, then placed on ice packs, and sent by overnight post to the Animal Health Diagnostic Center at Cornell University for analysis. ACTH measured with CIA. Reference interval for basal ACTH 9 – 35 pg/ml.

Outcome Measures:	ACTH concentrations at baseline and following TRH or domperidone in normal and PPID horses. A secondary outcome was basal ACTH measurements taken as part of the stimulation tests compared between PPID and non-PPID horses.
Main Findings:	Baseline ACTH concentrations were significantly higher when values for suspected and confirmed PPID cases were combined (median 52.4 pg/ml), compared to clinically normal horses (median 21.2 pg/ml) ($p<0.05$). Median basal ACTH for confirmed PPID cases (71.6 pg/ml) was higher than that of suspected PPID cases (24.2 pg/ml); however a statistical comparison of these data was not presented. Using at cut-off value of >36 pg/ml, baseline ACTH was reported to have: • sensitivity of 71%; specificity of 96%; diagnostic odds ratio (DOR) = 54 and diagnostic accuracy of 80% when confirmed PPID cases (n=25) were compared to clinically normal horses with normal histopathology (n=23) • sensitivity of 65%; specificity of 96%; DOR = 40 and diagnostic accuracy of 75% when confirmed and suspected PPID cases combined (n=35) were compared to clinically normal horses with normal histopathology (n=23) • sensitivity of 71%; specificity of 86%; DOR = 15 and diagnostic accuracy of 80% when confirmed PPID cases (n=25) were compared to all clinically normal horses (including those with normal histopathology, those with no post mortem and those with histologic pituitary changes; n=53) • sensitivity of 65%; specificity of 86%; DOR = 11 and diagnostic accuracy of 77% when confirmed and suspected PPID cases combined (n=35) were compared to all clinically normal horses (including those with normal histopathology, those with no post mortem and those with histologic pituitary changes; n=53)
Interpretation of Results:	Small sample size and splitting to subgroups for analysis reduces study power further. Different subgroups used for classifying animals based solely on clinical presentation, and for classification based on both clinical presentation and pituitary histopathology. Subgroup presentation of results is difficult to follow. Likely that at least a proportion of the study population were from a referral hospital population but information on sample selection limited therefore unable to assess selection bias and ability to generalise to other equine populations. Not all animals in study population included in ACTH element of study, and no details of case selection or reasons for exclusion are provided. Using control population selected based on absence of clinical signs of PPID (i.e. not suspected PPID cases) may introduce bias to estimates of diagnostic accuracy. Study uses histopathology, or a combination of histopathology and clinical diagnosis of PPID, as reference standard for diagnosis of PPID against various control subgroups. Seasonal

	effect on ACTH observed, but study did not use seasonally adjusted reference intervals. Basal ACTH was shown to have good sensitivity and specificity for diagnosis of PPID, when comparing PPID and normal horses.

Author, year: **Type of publication:**	Beech et al., 2011b Journal publication
Study Design:	Case control selection cross-sectional study; two-gate design using healthy controls
Aim/Objective of the Study:	To compare ACTH and cortisol responses to TRH administration in normal horses and in horses with PPID.
Setting:	Not stated
Study Population:	No information provided regarding study population selection. Substantial proportion of study population (ACTH data from 15 normal horses and 10 tests in 9 PPID horses) also included in authors' previous study (Beech et al., 2007). n=32 Controls: n=19 clinically normal horses (mixed breeds, no ponies) • no clinical signs of PPID ▫ of these animals, 7 had no histologic pituitary changes at post mortem, 7 had histologic pituitary hyperplasia, and 5 did not have a post mortem examination ▪ 11 geldings, 1 stallion, 7 mares; mean age 10.5±5.5 years (range 2-25 years) PPID cases: n=13 • 1 case receiving pergolide treatment • 8 horses and 2 ponies had ≥2 clinical signs of PPID ▫ of these animals, 3 had pituitary macroadenomas and 2 had microadenomas at post mortem, and 5 did not have a post mortem examination • 3 horses had mild signs of PPID ▫ of these animals, 1 had pituitary macroadenomas and 2 had microadenomas at post mortem • 2 geldings, 11 mares; mean age 22.2±6.5 years (range 13-34 years)
Diagnostic Test/Assay Investigated:	All TRH stimulation tests were performed by the same technician, and horses were maintained in familiar surroundings. Animals were catheterised 30 minutes prior to basal sample collection. Blood samples were collected in plastic EDTA tubes and centrifuged to separate plasma within 45 minutes of collection. Plasma was stored at -20°C in plastic tubes, then placed on ice packs, and sent by overnight post to the Animal Health Diagnostic Center at Cornell University for analysis. ACTH measured with CIA. Reference value for basal ACTH was <35 pg/ml. Control horses: • all controls had a single TRH stimulation test

	• 16 tested December-March, 1 in April, 1 in July, and 1 in August PPID cases: • 2 of the 13 cases had 3 TRH stimulation tests and all others had single test • 12 tests performed December-March, 2 in May, 1 in August, 1 in October and 1 in November
Outcome Measures:	Baseline ACTH concentrations, and ACTH and cortisol responses to TRH administration in normal and PPID horses.
Main Findings:	Elevated basal ACTH (>36 pg/ml): • PPID group: 10/17 tests from 13 horses • Control group: 1/19 horses Mean basal ACTH for PPID cases (78.0 pg/ml) significantly higher than that of clinically normal controls (21.0 pg/ml). Using at cut-off value of >36 pg/ml, baseline ACTH was reported to have: • sensitivity of 59% and specificity of 94%
Interpretation of Results:	Small sample size. Likely that at least a proportion of the study population were from a referral hospital population but information on sample selection limited therefore unable to assess selection bias and ability to generalise to other equine populations. Using control population selected based on absence of clinical signs of PPID (i.e. not suspected PPID cases) may introduce bias to estimates of diagnostic accuracy. Reference standard used for calculation of sensitivity and specificity of basal ACTH not clearly stated. Tests undertaken in different months but study did not use seasonally adjusted reference intervals. Interpretation of results challenging as some horses had multiple tests included in analysis and one PPID horse was receiving treatment with pergolide, although its ACTH levels were reported not be significantly different from the other PPID horses. Basal ACTH was shown to have had good specificity but moderate sensitivity for diagnosis of PPID, when comparing PPID cases with normal horses.

Author, year: **Type of publication:**	Couëtil et al., 1996 Journal publication
Study Design:	Case control selection cross-sectional study; two-gate design using healthy controls
Aim/Objective of the Study:	To compare basal ACTH concentrations between normal horses and horses with PPID. Secondary objectives were to determine whether ACTH concentrations differed between normal horses and ponies, and whether ACTH quantification in plasma was affected by blood sample handling techniques.
Setting:	University referral hospital
Study Population:	Study population comprised horses/ponies presented to a single university referral hospital over a 1-year period. n=49

	Controls: n=27 clinically normal animals (18 horses and 9 ponies) • determined to be normal on clinical examination, haematology and biochemistry • no recent history of corticosteroid or exogenous ACTH administration ▫ horses: 9 geldings/stallions, 9 mares; n=6 aged <10 years, n=5 aged 11-15 years, n=4 aged 16-20 years and n=3 aged >20 years; mean age 13.7 years ▫ ponies: 1 gelding/stallion, 8 mares; n=7 aged <10 years and n=2 aged 11-15 years; mean age 6.7 years PPID cases: n=22 (11 horses and 11 ponies) • n=18 clinically diagnosed based on hypertrichosis or abnormal hair coat shedding, plus ≥1 other clinical sign of PPID • n=4 with post mortem histological evidence of pituitary adenoma (including 1 horse with no clinical signs) ▫ horses: 6 geldings/stallions, 5 mares; n=1 aged <10 years, n=2 aged 11-15 years, n=1 aged 16-20 years and n=7 aged >20 years; mean age 21.5 years ▫ ponies: 5 geldings/stallions, 6 mares; n=1 aged 11-15 years, n=1 aged 16-20 years and n=9 aged >20 years; mean age 21.4 years
Diagnostic Test/Assay Investigated:	Blood sample collection performed between 9-12am, and horses/ponies stabled with access to water and hay during the sampling period. Blood samples were collected in EDTA glass tubes, kept chilled in iced water, then centrifuged at 1500g at room temperature for 10 minutes, within 15 minutes of sample collection. EDTA plasma then transferred into plastic tubes and stored frozen at -20°C and analysed within a week of sample collection. ACTH measured with a commercial human ACTH radioimmunoassay (RIA). Intra-assay and inter-assay variations, as were determined during the RIA validation.
Outcome Measures:	Baseline ACTH concentrations in healthy controls and PPID cases, and following different sample handling protocols.
Main Findings:	Mean basal ACTH concentrations: • Control group: ▫ horses 18.68 ± 6.79 pg/ml ▫ ponies 8.35 ± 2.92 pg/ml ▫ no statistically significant difference between age groups • PPID group: ▫ significantly higher than control animals ($p<0.001$) ▫ horses 199.18 ± 182.82 pg/ml ▫ ponies 206.21 ± 319.56 pg/ml ▫ no statistically significant difference between age groups Diagnostic cut-off points chosen arbitrarily as halfway between logarithmic mean ACTH concentration for PPID and control groups: horses 50 pg/ml and ponies 26.96 pg/ml. Using these cut-off values, basal ACTH was reported to have: • sensitivity of 90.9% and specificity of 100% in horses

	• sensitivity of 81.8% and specificity of 100% in ponies
Interpretation of Results:	Small sample size. Limited information regarding selection of cases and controls, although sample selection likely to introduce bias. Inclusion criteria for PPID group likely to have selected for cases with advanced disease. Using control population selected based on absence of clinical signs of PPID (i.e. not suspected PPID cases) may introduce bias to estimates of diagnostic accuracy. Clinical signs (hypertrichosis or abnormal hair coat shedding, plus ≥1 other clinical sign of PPID) used as reference standard for calculation of sensitivity and specificity of basal ACTH, with the exception of one case without hypertrichosis that had post mortem confirmation of pars intermedia adenoma. Sensitivity and specificity calculated based on the markedly different diagnostic cut-off points derived for horses and ponies, and no overall measures of diagnostic test accuracy are presented. Study conducted over a 1-year period but did not use seasonally adjusted reference intervals. Basal ACTH was shown to have had good sensitivity and excellent specificity for diagnosis of PPID, when comparing PPID cases with normal horses/ponies.

Author, year: **Type of publication:**	Horn et al., 2020 Journal publication
Study Design:	Case control selection with repeated cross-sectional studies; two-gate design using healthy controls
Aim/Objective of the Study:	To compare measures of diagnostic accuracy of baseline and post-TRH stimulation plasma ACTH concentrations in horses using diagnostic cut-off values and reference intervals
Setting:	Not stated
Study Population:	No information provided regarding study population selection. Study population were enrolled over a 24 month period, and each horse was followed for up to 12 months. n=106 horses (aged ≥10 years; ponies were excluded); all considered to be healthy other than clinical signs of PPID amongst cases. Enrolled horses had not received pergolide treatment in the 12 months prior to the study, and had not travelled within the 24 hours prior to sampling. • 53 geldings, 4 stallions and 49 mares • breeds included were: Australian Stock Horse (n=35), Standardbred (n=24), Warmblood (n=18), Thoroughbred (n=12), Arab (n=7), Quarter Horse (n=4), and mixed breeds (n=6) Controls: n=72 clinically normal horses • no clinical signs of PPID during the study • outlying values for basal or 30 minute post-TRH ACTH concentration returned from ≤2 monthly samples in the 12

	month period (not stated, but presumably this criterion applied solely to high outlying values) • in subset where post mortem examination was undertaken, findings not consistent with pituitary adenoma (number of controls with post mortem not reported) ▫ median age 16 years (range 10 – 27 years) PPID cases: n=34 horses, with ≥2 of the following 3 criteria: • clinical signs consistent with PPID (hypertrichosis or delayed shedding and epaxial muscle wastage, abnormal fat distribution or abnormal sweating) • post mortem findings confirming or excluding pituitary adenoma or hyperplasia • outlying values for basal or 30 minute post-TRH ACTH concentration returned from ≥6 monthly samples in the 12 month period ▫ median age 22 years (range 11 – 31 years); significantly greater than control group (p<0.001)
Diagnostic Test/Assay Investigated:	Horses were sampled once monthly (during the second week of each month) for up to 12 months. Samples were collected with horses in familiar surroundings (either home premises or in paddocks at the institution where the study was undertaken), with ad libitum access to food and water prior to and following sampling. Samples were collected by venipuncture (horses were not catheterised) at baseline and at 30 minutes following TRH administration. Blood was collected into plastic EDTA vacutainer tubes, then kept on ice, centrifuged and separated within 2 hours of collection. ACTH measured using CIA (Immulite 1000 assay) within 8 hours of collection.
Outcome Measures:	Sensitivity, specificity, positive likelihood ratios and diagnostic accuracy (area under the ROC curve) of ACTH compared to reference standard comprising the eligibility criteria used to define PPID cases. Youden's index used to derive monthly ACTH cut-off values that optimise differentiating ability (between diseased and non-diseased animals) when equal weight is given to sensitivity and specificity. Monthly reference intervals for ACTH were derived from the control population using Box-Cox transformation and bootstrapping.
Main Findings:	Youden's index derived monthly ACTH diagnostic cut-off values ranged from a minimum of 24.0pg/ml in June (winter in Southern hemisphere) to a maximum of 79.1pg/ml in April (autumn in Southern hemisphere). The upper limit of derived monthly reference intervals ranged from a minimum of 28.6pg/ml in October, based on 42 control horses, to a maximum of 138.0pg/ml in March, based on 50 control horses. Using derived the upper limit of derived reference intervals resulted in lower sensitivity and higher specificity for basal ACTH compared to using the derived diagnostic cut-off values (p=0.002 and p=0.01, respectively).

	Greatest sensitivity identified in April (autumn), using cut-off value of 79.1pg/ml. Using this cut-off value, basal ACTH was reported to have: • sensitivity of 100% (95% CI 64.6-100%) and specificity of 95.4% (95% CI 84.5-99.2%), positive likelihood ratio = 21.5 (95% CI 4.98-56.0) and area under ROC curve 0.99 (n=7 PPID cases and n=44 controls) Greatest specificity identified in May (autumn), using cut-off value of 41.6pg/ml. Using this cut-off value, basal ACTH was reported to have: • sensitivity of 88.9% (95% CI 56.5-99.4%) and specificity of 97.8% (95% CI 88.7-99.9%), positive likelihood ratio = 40.9 (95% CI 5.8-281.0) and area under ROC curve 0.93 (n=9 PPID cases and n=46 controls) Lowest sensitivity identified in November (spring) and December (summer). Only November data extracted here due to slighter larger sample size included. Using the November cut-off value of 25.7pg/ml, basal ACTH was reported to have: • sensitivity of 80.0% (95% CI 49.0-96.5%) and specificity of 72.5% (95% CI 57.2-83.9%), positive likelihood ratio = 2.9 (95% CI 1.62-5.20) and area under ROC curve 0.84 (n=10 PPID cases and n=41 controls) Lowest specificity identified in March (autumn), using cut-off value of 58.3pg/ml. Using this cut-off value, basal ACTH was reported to have: • sensitivity of 85.7% (95% CI 48.7-99.3%) and specificity of 63.3% (95% CI 49.3-75.3%), positive likelihood ratio = 2.3 (95% CI 1.45-3.75) and area under ROC curve 0.82 (n=7 PPID cases and *n*=50 controls)
Interpretation of Results:	ACTH cut-off values and reference intervals derived and these values were then used to assess diagnostic test accuracy. Variable sample size per month (between 49 and 57 horses in total per month) included in data analyses. Seasonal variations seen may not be directly comparable to the climate in the northern hemisphere. Eligibility criteria for defining PPID cases used as reference standard for calculation of sensitivity and specificity of basal ACTH, effectively forming a composite reference standard. However, not all PPID cases had each of the 3 eligibility criteria, and no details regarding the frequency of clinical signs, post mortem pituitary abnormalities or recurrent outlying ACTH values are reported. Using control population selected based on absence of clinical signs of PPID (i.e. not suspected PPID cases) may introduce bias to estimates of diagnostic accuracy. Horses with outlying ACTH results occurring on 3-5 occasions during the 12 month study period were excluded. Results reported separately for each month, and no overall measures of diagnostic test accuracy are presented. Basal ACTH was shown to have had good sensitivity and specificity for diagnosis of PPID, when comparing PPID cases horses without clinical signs of PPID. Sensitivity for basal ACTH was greater where

	derived diagnostic cut-off values were used to define a positive result, whereas the higher ACTH values derived as the upper limit of monthly reference intervals provided greater specificity.

Author, year: **Type of publication:**	Horowitz et al., 2003 Conference abstract
Study Design:	Case control selection cross-sectional study; two-gate design using healthy controls
Aim/Objective of the Study:	To compare basal α-MSH, β-endorphin and ACTH concentrations between normal horses and horses with PPID, and to determine sensitivity and specificity of these values for diagnosis of PPID when compared to the ODST.
Setting:	Not stated
Study Population:	No information provided regarding study population selection. n=63, classified as PPID cases or normal controls based on the results of ODST Controls: n=38 age-matched controls; mean age 22.5±2.9 years PPID cases: n=25; mean age 21.3±4.7 years
Diagnostic Test/Assay Investigated:	Blood was collected in EDTA tubes and stored at -80°C prior to analysis. ACTH measured at the Michigan State Endocrinology Laboratory using RIA.
Outcome Measures:	ACTH tests results compared to ODST diagnosis. ROC curve used to derive ACTH cut-off value that optimised sensitivity and specificity.
Main Findings:	Mean basal ACTH concentrations: • Control group: ▫ 8.0 ± 9.1 pmol/l (equivalent to 36.3 ± 41.3 pg/ml) • PPID group: ▫ significantly higher than control animals ($p<0.001$) ▫ 33.7 ± 23.5 pmol/l (equivalent to 153.0 ± 106.7 pg/ml) Using a diagnostic cut-off point of 11 pmol/l (equivalent to 49.95 pg/ml), derived from ROC curve analysis, basal ACTH was reported to have: • sensitivity of 84% and specificity of 89.5% α-MSH and β-endorphin significantly higher in PPID group compared to controls (both $p<0.001$). Both α-MSH and β-endorphin had a sensitivity of 88% and specificity of 84.2%.
Interpretation of Results:	Conference abstract with limited information provided. Small sample size. No description of sample selection, nor clinical signs exhibited by included animals therefore unable to determine if study population is appropriate for a diagnostic test accuracy study. Unable to determine whether results are generalisable to other populations. ODST used as reference standard for calculation of sensitivity and specificity of basal ACTH. Basal ACTH was shown to have had good sensitivity and specificity for diagnosis of PPID, when comparing PPID cases with normal horses.

Author, year: **Type of publication:**	McGowan et al., 2013b Journal publication
Study Design:	Cohort selection cross-sectional study; single gate
Aim/Objective of the Study:	To evaluate basal α-MSH and ACTH concentrations for the diagnosis of PPID in a population of horses aged ≥15 years
Setting:	Field based
Study Population:	Study population were selected from a subset of animals in a preceding cross-sectional study of geriatric horses. n=974 horses (aged ≥15 years) recruited via a questionnaire from members of an equestrian organisation, from which n=340 subgroup selected by geographic location for veterinary clinical examination and endocrine tests (n=339). Of this subgroup, n=325 had ACTH assay performed, of which 304 included in final analysis for ACTH n=21 with owner-reported history of hypertrichosis with ≤2 other clinical signs of PPID excluded from analysis Controls: n=281 had no clinical evidence of PPID, of which 280 included in final analysis for ACTH PPID cases: n=27 had hypertrichosis plus ≥3 other current and/or historical clinical signs of PPID (based on both veterinary clinical examination findings and owner-reported questionnaire data), of which 24 included in final analysis for ACTH
Diagnostic Test/Assay Investigated:	Blood samples collected prior to moving the horse and clinical examination, to minimise stress or excitement. Blood was collected into glass EDTA vacutainer tubes, then immediately centrifuged at 1000g for 10 minutes using a portable centrifuge. EDTA plasma was then placed on ice packs before storage at -80°C (within 6 hours) until being shipped frozen to the laboratory for analysis (1-24 months after sample collection). ACTH measured using CIA (Immulite 1000 assay).
Outcome Measures:	Sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios of ACTH compared to reference standard of hypertrichosis plus ≥3 other clinical signs of PPID (based upon results of one-way ANOVA comparing α-MSH and ACTH concentrations in horses with owner-reported hypertrichosis and 0-12 other clinical signs). Youden's index used to derive ACTH cut-off value that optimises differentiating ability (between diseased and non-diseased animals) when equal weight is given to sensitivity and specificity. PPID prevalence estimate of 13%, based owner-reported hypertrichosis, used for calculation of ACTH sensitivity, specificity, predictive values and likelihood ratios.
Main Findings:	ACTH showed a moderate positive correlation (r=0.69; p<0.001) with total number of clinical and historical indicators of PPID, in animals with owner-reported hypertrichosis. Youden's index derived diagnostic ACTH cut-off values: Non-autumn seasons: 29.7pg/ml

	Autumn: 77.4pg/ml Using these cut-off values, basal ACTH was reported to have: • sensitivity of 80.0% and specificity of 82.5% in non-autumn seasons (June-February) (n=20 PPID cases and n=217 controls) • sensitivity of 100% and specificity of 95.2% in autumn (March-May) (n=4 PPID cases and n=63 controls)
Interpretation of Results:	ACTH cut-off values derived in a field-based population and these values were then used to assess diagnostic test accuracy. Samples were collected in field conditions but confined to a geographically convenient area. Small sample size of animals samples during autumn. Seasonal variations seen may not be directly comparable to the climate in the northern hemisphere. Clinical signs (hypertrichosis plus ≥3 other clinical signs of PPID) used as reference standard for calculation of sensitivity and specificity of basal ACTH. Inclusion criteria for PPID group likely to have selected for cases with advanced disease. Using control population selected based on absence of clinical signs of PPID (i.e. not suspected PPID cases) may introduce bias to estimates of diagnostic accuracy. Animals with hypertrichosis plus ≤2 other clinical signs of PPID were excluded. Results reported separately for autumn and non-autumn period, and no overall measures of diagnostic test accuracy are presented. Basal ACTH was shown to have had good sensitivity and specificity for diagnosis of PPID, when comparing PPID cases with geriatric horses/ponies without clinical signs of PPID. Measures of diagnostic test accuracy for basal ACTH were greater during the autumn compared to other seasons combined.

Author, year: **Type of publication:**	Perkins et al., 2002 Journal publication
Study Design:	Case control selection cross-sectional study (for component of study evaluating measures of ACTH diagnostic test accuracy); two-gate design using combination of healthy controls combined with suspected cases
Aim/Objective of the Study:	To validate the CIA for ACTH and to calculate the sensitivity and specificity of plasma ACTH levels for detecting PPID.
Setting:	New York State Diagnostic Laboratory Population for evaluating ACTH sensitivity and specificity from university referral hospital.
Study Population:	Retrospective review of referral hospital clinical records from April 1997 to December 1998, to collate clinical data for animals that had ACTH samples within that period. Data collected included age, month of sampling, ACTH concentration, hypertrichosis, laminitis, lethargy and other signs of PPID, plus any other diagnosis. Histologic evidence of pituitary hyperplasia was recorded for cases undergoing post mortem examination (n=6). n=68 Controls: n=29

	• no clinical signs consistent with PPID or had alternative definitive diagnosis that explained clinical signs ▫ age and sex data only reported combined with possible PPID group; overall median age 16 years (range 2-21 years) PPID cases: n=19 • based on hypertrichosis ▫ 9 mares, 2 stallions and 8 geldings; median age 21 years (range 13-32 years) ▫ breed data appear to be reported erroneously: text results state 2 of 5 ponies had hypertrichosis, yet table displays 0% for pony breed in PPID group Possible PPID cases: n=20 • clinical signs consistent with PPID (listed as recurrent laminitis, lethargy, obesity, secondary infections, polyuria/polydipsia and muscle wastage), but not hypertrichosis ▫ age and sex data only reported combined with control group; overall median age 16 years (range 2-21 years)
Diagnostic Test/Assay Investigated:	No information regarding blood sampling or sample handling reported. ACTH assumed to be measured with CIA (Immulite), since it was the assay validated in another phase of this study. Laboratory's previously derived diagnostic cut-off value for PPID was used (>35 pg/ml; based on data from young-mature horses and ponies). The intra-assay coefficient of variation was determined on samples for each of 4 equine plasma samples (mean 9.3%). Inter-assay precision on 2 equine plasma samples assayed for 25 consecutive runs over a month (9.1% for low control and 7.0% for high control).
Outcome Measures:	Basal ACTH concentration
Main Findings:	Elevated ACTH significantly associated with PPID group (defined based on hypertrichosis; $p<0.01$). 'Winter onset', defined as samples obtained between October-April, was not associated with elevated ACTH ($p=0.88$). Of the 6 animals that had post mortem examination, 4 cases had no gross evidence of pituitary enlargement and all had normal ACTH level, while the remaining 2 had histological evidence of pituitary adenomatous hyperplasia, of which one had normal ACTH (on several occasions) but did have hypertrichosis and severe hyperhidrosis. Which group these 6 animals were in is not reported. Using a diagnostic cut-off point of >35 pg/ml, basal ACTH was reported to have: • sensitivity of 84% (95% CI 60-97%) and specificity of 78% (95% CI 63-88%)
Interpretation of Results:	First phase of the study demonstrated the ACTH was valid for use in horses. Insufficient information provided to assess methods used by laboratory to derive cut-off value for ACTH. Small sample size. Subject to inherent biases affecting retrospective studies, including potential for missing data or data not accurately recorded in clinical

	records. Hypertrichosis used as sole criterion used for clinical reference standard. An alternative definitive diagnosis to explain clinical signs may not have been sufficient to exclude PPID cases from the non-PPID control group. Using control population selected based on absence of clinical signs of PPID (i.e. not suspected PPID cases) may introduce bias to estimates of diagnostic accuracy. Study conducted over a 21-month period but did not use seasonally adjusted reference intervals. Basal ACTH had good sensitivity and specificity for diagnosis of PPID. Sensitivity and specificity presumed to be against non-PPID control and possible PPID groups combined, however this is not stated and no raw data are presented.

Author, year: **Type of publication:**	Rendle et al., 2015a Journal publication
Study Design:	Case control selection cross-sectional study; two-gate design using healthy controls
Aim/Objective of the Study:	To determine whether the mean of two paired measurements of ACTH concentration is more reliable in assessing PPID than a single measurement.
Setting:	Two university referral hospitals (USA and Australia) and one private referral hospital (UK).
Study Population:	Retrospective data collection from review of referral hospital clinical records and prospective data collection from research herds. n=250; median age 16 years (range 3-33 years) Controls: n=50 horses (n=63 paired samples) • no clinical suspicion of PPID PPID cases: n=76 horses (n=90 paired samples) • receiving treatment with pergolide Possible PPID cases: n=124 horses (n=148 paired samples) • being investigated for PPID Histological examination was performed for n=67 untreated horses, by board-certified pathologists at a single institution, and PPID was diagnosed where there was par intermedia adenomatous hyperplasia, microadenoma or macroadenoma. • n=44 horses had histological changes • n=23 horses considered to have normal pars intermedia Histology used as reference standard for evaluation of ACTH diagnostic test accuracy.
Diagnostic Test/Assay Investigated:	Paired blood samples collected 5-15 minutes apart, then chilled and centrifuged. Plasma was then refrigerated and analysed within 24 hours or frozen at -20°C or -80°C for up to 4 weeks prior to analysis. Results of analysis of the first sample were used as the single sample result. Used seasonally adjusted reference intervals of <29pg/ml for non-autumn (November-July) and <47pg/ml for autumn (August-October). It is not reported, but presumably autumn months were altered accordingly for samples obtained in Australia.

	ACTH measured with CIA (Immulite). The coefficient of variation was determined over a range of concentrations as 6%, and for values between 20 and 39 pg/ml, the median coefficient of variation was 9.0%.
Outcome Measures:	Agreement between single and paired ACTH samples for diagnosis of PPID, based on seasonally adjusted reference intervals. ROC curve used to derive ACTH cut-off values for single and paired samples that optimised overall diagnostic accuracy (% correctly classified). Sensitivity and specificity for ACTH calculated for both single and paired measurements.
Main Findings:	Of the 211 samples from untreated horses (both PPID positive and negative), single and paired tests were in agreement in 205 cases (97.2%). All 6 cases in which results were not in agreement were tested in the non-autumn period, and close to the cut-off point (median 29.2pg/ml). Single ACTH measurements did not agree with histopathology results in 31.3% of cases (n=21/67; 19 false negatives and 2 false positives): ACTH for these cases n=1 >39pg/ml; n=9 20-39pg/ml and n=11 <20pg/ml. *Not reported, but calculated from these data, basal ACTH would have:* • *sensitivity of 56.8% (95% CI 41.0-71.7%) and specificity of 91.3% (95% CI 72.0-98.9%)* For single samples: Using a diagnostic cut-off point of >21.3 pg/ml (selected to give greatest diagnostic accuracy), basal ACTH was reported to have: • sensitivity of 69.4% and specificity of 80.9% Using a diagnostic cut-off point of >23.6 pg/ml, basal ACTH was reported to have: • sensitivity of 61.1% and specificity of 95.2% Mean of paired ACTH measurements did not agree with histopathology results in 32.8% of cases (n=22/67; 20 false negatives and 2 false positives): mean ACTH for these cases n=1 >39pg/ml; n=10 20-39pg/ml and n=11 <20pg/ml. *Not reported, but calculated from these data, mean paired ACTH would have:* • *sensitivity of 54.6% (95% CI 38.9-69.6%) and specificity of 91.3% (95% CI 72.0-98.9%)* For paired samples: Using a diagnostic cut-off point of >21.9 pg/ml (selected to give greatest diagnostic accuracy), mean ACTH was reported to have: • sensitivity of 72.2% and specificity of 76.2% Using a diagnostic cut-off point of >23 pg/ml, mean ACTH was reported to have: • sensitivity of 66.7% and specificity of 85.7%
Interpretation of Results:	Histopathology was only available for subset of cases. Limited information provided on selection of study sample and no details of clinical signs, therefore it is not possible to determine whether the

	control group (no histologic evidence of PPID) presented with clinical signs. Subject to inherent biases affecting retrospective studies, including potential for missing data or data not accurately recorded in clinical records. No information is provided regarding which of the 2 groups (being investigated for PPID or no clinical suspicion of PPID) that the 67 horses with post mortem histology were in. Details of time interval between ACTH measurement and post mortem examination for histopathology not reported. Study uses histopathology as reference standard for diagnosis of PPID. Cut-off values used for initial comparison with histopathology not clearly stated, but presumed to be based on the seasonally adjusted reference intervals provided in the methods section. The season(s) in which the 67 cases were sampled is not reported. Sensitivity and specificity of single and paired samples using the cut-off points based on the seasonally adjusted reference intervals are not reported (however these were calculated from raw data presented). The vast majority of cases with histologic evidence of PPID incorrectly classed as negative on single ACTH measurement had equivocal results close to the non-autumn cut-off value (29 pg/ml). Rationale for selecting a cut-off value ~23 pg/ml for both single and paired samples not reported. Using the mean value of paired ACTH tests did not have a diagnostic benefit over a single basal sample. Basal ACTH had relatively low sensitivity and moderate-good specificity for diagnosis of PPID at cut-off points selected to optimise overall diagnostic accuracy, which are considerably lower than those used by the participating institutions.

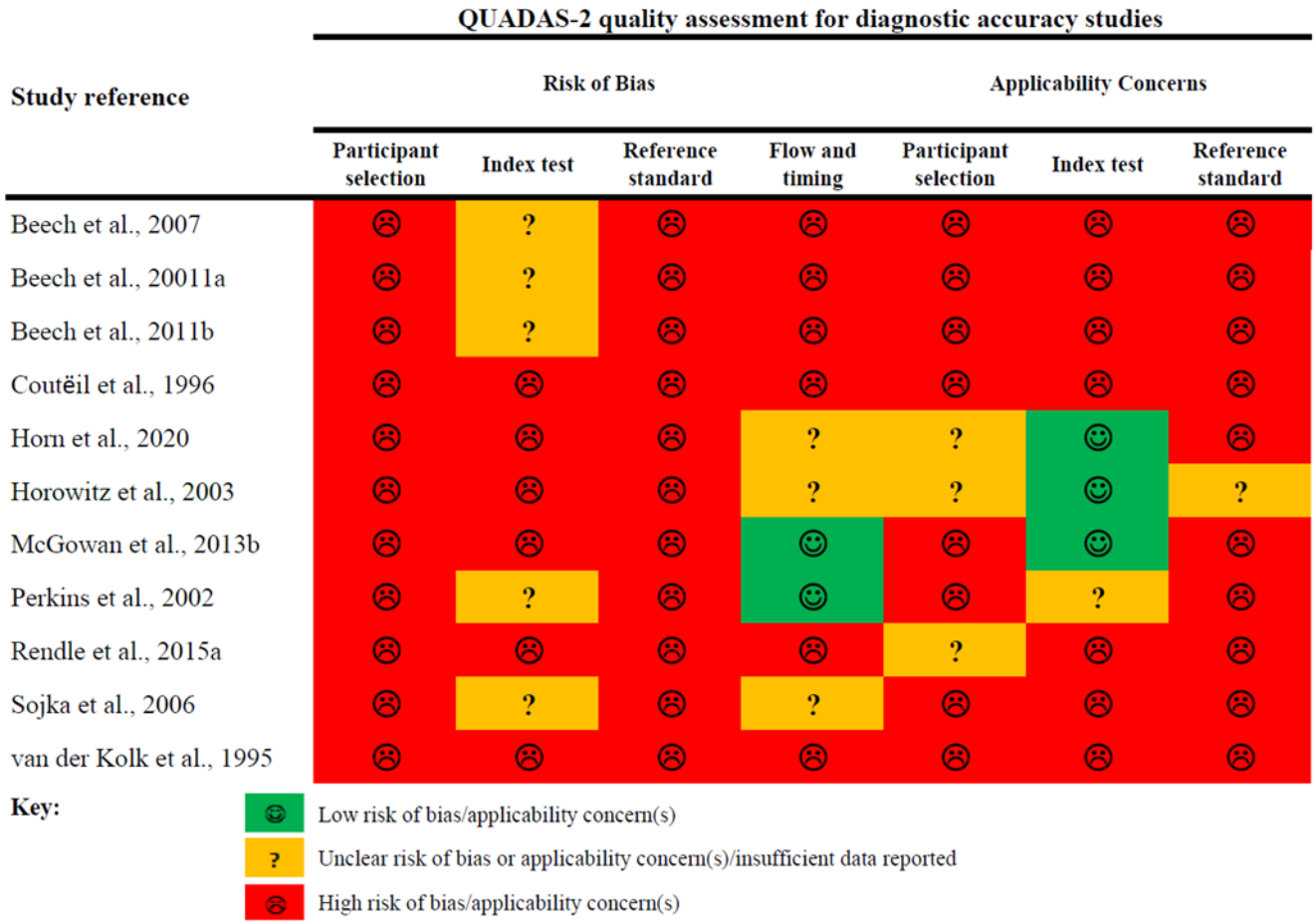
Author, year: **Type of publication:**	Sojka et al., 2006 Conference abstract
Study Design:	Case control selection cross-sectional study; two-gate design using healthy controls
Aim/Objective of the Study:	To investigate the effect of domperidone on ACTH concentrations in horses with and without histologically-confirmed tumours of the pars intermedia.
Setting:	Not stated
Study Population:	No information provided regarding study population selection. n=7 Controls: n=2 • average age 14.5 years (12 and 17 years) PPID cases: n=5 • average age 22.2 years (range 17-35 years)
Diagnostic Test/Assay Investigated:	Blood samples were collected into silicone-coated EDTA tubes and plasma was separated within 15 minutes of collection and frozen prior to analysis. Further blood samples were obtained 4 and 8 hours following oral administration of 1.5g domperidone. ACTH measured using CIA.
Outcome Measures:	ACTH response following administration of domperidone. Secondary outcome was comparison of basal ACTH concentrations with gross and/or histologic evidence of pars intermedia adenoma.

Main Findings:	Basal ACTH concentrations: • Control group: ▫ 20.5 and 37.1 pg/ml (mean 28.8 pg/ml) • PPID group: ▫ 37.8, 25.8, 30.8, 271.0, and 77.0 pg/ml (mean 77.5 ± 104.0 pg/ml) ▫ 3/5 PPID cases had baseline ACTH values within reference interval (10-59 pg/ml) therefore would not have been diagnosed with PPID *Not reported, but calculated from these data, using a cut-off value of >59 pg/ml basal ACTH would have:* • *sensitivity of 40.0% (95% CI 5.3-85.3%) and specificity of 100% (95% CI 15.8-100%)* *Using a cut-off value of >29 pg/ml (upper limit of non-autumn reference interval determined by Copas & Durham, 2012), 4/5 cases with histologic changes and 1/2 controls would have been diagnosed with PPID, and basal ACTH would have:* • *sensitivity of 80.0% (95% CI 28.4- 99.5%) and specificity of 50.0% (95% CI 1.2-98.7%)*
Interpretation of Results:	Conference abstract with limited information provided. Very small sample size with no details of case selection or clinical signs provided, therefore it is not possible to determine whether the control group (no histologic evidence of PPID) presented with clinical signs. Unable to determine whether results are generalisable to other populations. Details of time interval between ACTH measurement and post mortem examination for histopathology not reported. Study uses histopathology as reference standard for diagnosis of PPID. Time of year samples taken not reported and single cut-off value of >59 pg/ml used for ACTH diagnosis of PPID. Sensitivity and specificity are not reported (however these were calculated from raw data presented). Two of the five cases with histologic evidence of PPID classed as negative on basal ACTH measurement had equivocal results. Using a cut-off value of >59 pg/ml, basal ACTH had low sensitivity and 100% specificity, when comparing PPID cases to two control animals without pars intermedia adenomas.

Author, year: **Type of publication:**	van der Kolk et al., 1995 Journal publication
Study Design:	Case control selection cross-sectional study; two-gate design using healthy controls
Aim/Objective of the Study:	To determine the sensitivity of measuring basal plasma ACTH concentration for the detection of pars intermedia adenoma (plasma glucose, cortisol and insulin, as well as urinary concentration of corticoids and urinary corticoid:creatinine ratio were also evaluated, but are not reported here as not relevant to systematic review).
Setting:	University referral hospital

Study Population:	PPID cases examined between February 1990 and November 1993 identified from review of referral hospital clinical records. Controls: n=7 • clinically healthy Dutch Warmbloods ▫ 4 geldings, 3 mares; mean age 10 ± 1.5 years (range 7-17 years) PPID cases: n=24 • 11 Dutch Warmbloods and 13 ponies ▫ 12 geldings, 12 mares; mean age 20 ± 1.2 years (range 12-30 years) • presented for investigation of suspected PPID due to presence of hypertrichosis • all had histologically confirmed pituitary pars intermedia adenomas ▫ n=16 cases had basal ACTH measured
Diagnostic Test/Assay Investigated:	Blood samples collected in EDTA tubes at 9am, then centrifuged at 1500g for 10 minutes at 4°C. Plasma was separated and stored as 2ml samples at -20°C prior to analysis. ACTH measured with RIA. Intra-assay and inter-assay coefficients of variation ACTH were 8% and 12%, respectively. Mean ACTH plus 2 x standard deviation from control horses used to diagnostic determine cut-off value.
Outcome Measures:	Basal ACTH concentrations in healthy controls and cases presenting with hypertrichosis and being investigated for PPID. Sensitivity of ACTH for detecting pars intermedia adenoma.
Main Findings:	Basal ACTH concentrations: • Control group: ▫ 30 ± (standard error) 5.0 pg/ml (range 10-50 pg/ml) ▫ cut-off value of 55 pg/ml calculated • PPID group (n=16/24): ▫ 489 ± 84.8 pg/ml (range 104-1000 pg/ml) Using a diagnostic cut-off point of >55 pg/ml, and 16 PPID cases, basal ACTH was reported to have: • sensitivity of 100%
Interpretation of Results:	Small sample size and no explanation provided as to why basal ACTH measurements were not available for 8 of the 24 PPID cases. PPID cases all from referral hospital population, and no details provided regarding selection of control population. Details of time interval between ACTH measurement and post mortem examination for histopathology not reported. Using control population selected based on absence of clinical signs of PPID (i.e. not suspected PPID cases) may introduce bias to estimates of diagnostic accuracy. Study uses histopathology as reference standard for diagnosis of PPID. Diagnostic cut-off determined by mean plus 2 x standard deviation from small sample of clinically normal animals; however data are presented as means ± standard error. Time of year samples taken not reported and single cut-off value used for ACTH diagnosis of PPID. Study design meant only sensitivity could be calculated for ACTH. Using a cut-off value of >55 pg/ml, basal ACTH had 100% sensitivity for identifying PPID cases.

Appendix 3: QUADAS-2 assessment for diagnostic accuracy studies



Chapter 4

Efficacy of pergolide for the management of equine pituitary pars intermedia dysfunction:

A systematic review

Efficacy of pergolide for the management of equine pituitary pars intermedia dysfunction: A systematic review

The following chapter collates and reviews literature pertaining to the treatment of PPID with pergolide, and is presented as it was published in The Veterinary Journal.

Reference: Tatum RC, McGowan CM, Ireland JL (2020). Efficacy of pergolide for the management of equine pituitary pars intermedia dysfunction: A systematic review. The Veterinary Journal 266: 105562.

Summary

Pergolide, a dopamine agonist, is commonly administered to manage pituitary pars intermedia dysfunction (PPID), a progressive neurodegenerative disease prevalent in aged horses. However, available evidence regarding pergolide's efficacy in improving clinical and endocrine parameters is limited. The aim of this systematic review was to assess published literature and evaluate evidence regarding whether pergolide treatment results in improvement of clinical signs and/or adrenocorticotrophic hormone (ACTH) concentration compared to no treatment or other unlicensed treatments. Systematic searches of electronic databases were undertaken in April 2019, repeated in August and October 2019, and updated in July 2020. English language publications published prior to these dates were included. Screening, data extraction and quality assessment of publications was undertaken individually by the authors using predefined criteria and subsequently cross-checked. Modified critically appraised topic data collection forms were used to extract data. Due to marked between-study variations, meta-analysis was not undertaken.

After removal of duplicate records; 612 publications were identified, of which 129 abstracts were screened for eligibility and 28 publications met criteria for inclusion in the review. Most studies were descriptive case series, cohort studies or non-randomised, uncontrolled field trials. Despite marked variation in study populations, case selection, diagnostic protocols, pergolide dose, follow-up period and outcome measures, in the vast majority of the included studies, pergolide was reported to provide overall clinical improvement in >75% of cases. However, reported improvements in individual clinical signs varied widely. A reduction in plasma ACTH concentrations was reported in 44-74% of cases, while normalisation to within reported reference intervals occurred in 28-74% of cases.

Introduction

Pituitary pars intermedia dysfunction (PPID) is the most prevalent endocrine disorder of older horses (aged ≥15 years; McGowan et al., 2013a). This demographic now represents a significant proportion of the equine population (Mellor et al., 2001; Brosnahan and Paradis, 2003b; Ireland et al., 2011a). Consequently, PPID is diagnosed with increasing frequency (Rohrbach et al., 2012) and is therefore important in equine practice. PPID is a progressive neurodegenerative disease, possibly caused by oxidative stress, which affects the inhibitory dopaminergic hypothalamic neurones, leading to a loss of dopaminergic control (McFarlane and Cribb 2005; McFarlane et al., 2005c; McFarlane, 2007). Consequently, a lack of regulation within the melanotrope cells of the pituitary pars intermedia results in over production of multiple pro-opiomelanocortin (POMC)-derived peptides and their derivatives, including adrenocorticotrophic hormone (ACTH; Heinrichs et al., 1990). The precise consequences of this are not fully understood (Durham, 2016a). However, once present, PPID is a progressive lifelong condition with varying clinical signs (Schott, 2002; McGowan, et al., 2013a; Ireland and McGowan, 2018). Currently, measurement of basal plasma ACTH concentration is widely used for diagnosing and monitoring PPID and is considered sufficient when seasonal reference intervals are utilised (McGowan et al., 2013b; Durham et al., 2014).

Pergolide mesylate, an ergot-derived, long-acting dopamine D2 receptor agonist, is used extensively to manage the disease (Anon, 2011a; Durham et al., 2014). It was previously used as an adjunctive treatment for Parkinson's disease (Van Camp et al., 2004), an age-associated neurodegenerative condition in humans, which has been compared to PPID (McFarlane, 2007). Horses with PPID have been found to have significantly less dopamine and dopamine metabolites compared to normal controls (Millington et al., 1988) and systemic supplementation of dopamine or a dopamine agonist was shown to decrease plasma concentration of POMC peptides (Orth et al., 1982). Pergolide is reportedly well tolerated, rapidly absorbed and reaches high plasma concentrations in horses (Gehring et al., 2010; Rendle et al., 2019). Therefore, the rationale for the use of the dopamine agonist pergolide for the management of PPID is strong. However, systematic evaluation of treatment options for PPID has not been undertaken and treatment recommendations have evolved primarily from clinical experience.

Systematic reviews are an established reproducible method of collating and assessing available evidence to answer a specific research question. They provide a high level of evidence and can be used to assess interventions (Anon 2011b; Higgins et al., 2019), helping to inform decisions about the provision of healthcare (Rodgers et al., 2009). The aim of this paper was to perform the first systematic review of published literature about the efficacy of pergolide for the treatment of PPID in horses and ponies. Unless otherwise stated, all results presented in this paper pertain to horses and/or ponies and may not be applicable to other equids. The question addressed by this review was: 'in horses/ponies diagnosed with PPID, is treatment with pergolide effective in improving clinical signs and/or plasma ACTH concentrations compared to no treatment or other unauthorised treatments?'. Published literature regarding the use of pergolide to treat PPID was systematically reviewed and included studies are presented with assessment of their design and validity (Moher et al., 2009).

Materials and methods

Search methodology

The search strategy was based on current guidelines (McGowan et al., 2016). A systematic search of electronic databases was undertaken in April 2019, repeated in August and October 2019 and updated in July 2020. In order to obtain the best coverage of the veterinary literature, databases searched were; NCBI PubMed, Clarivate Analytics Web of Science, CAB Direct, SciVerse Scopus, and the database of the International Veterinary Information Service (IVIS; Grindlay et al., 2012). Additional relevant records were identified via the bibliographies of retrieved publications. Searches were conducted using a range of free text search terms and MEDLINE MeSH terms, including (Equine Cushing's syndrome), (pituitary pars intermedia dysfunction) and (treatment). The full list of search terms and Boolean operators is detailed in Appendix 1. This review focused on PPID in horses and ponies, therefore search terms relating to donkeys and other equids were not included. All retrieved records were imported and formatted into a predesigned Microsoft Excel spreadsheet.

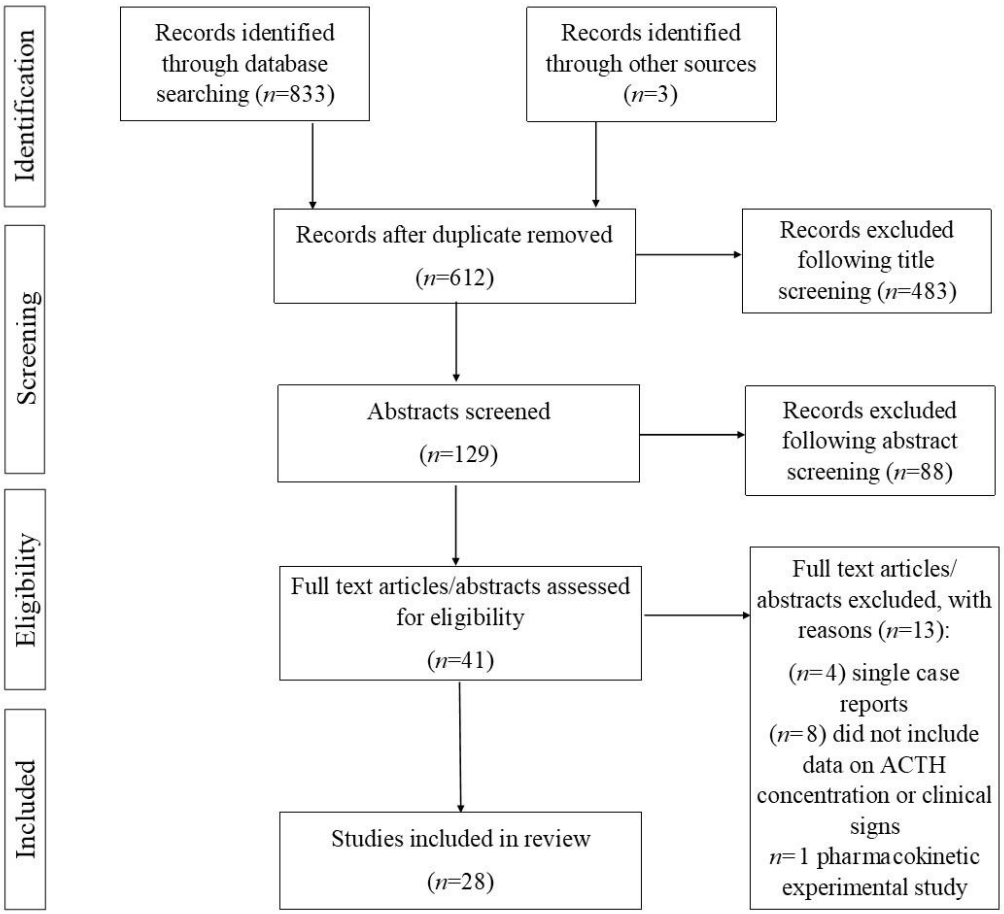


Figure 1: Flow diagram of the search strategy undertaken in a systematic review to identify and screen publications with information on the effect of pergolide treatment on clinical signs and plasma adrenocorticotrophic hormone (ACTH) concentrations in horses and ponies with pituitary par intermedia dysfunction (PPID), resulting in the identification of 28 included publications (adapted from Moher et al., 2009).

Inclusion criteria

Due to a lack of randomised controlled trials, studies of Level 4 evidence and above were included (Anon, 2011b). Descriptive individual case reports with no comparison group were excluded. Limitations on publication date, setting or study population were not imposed. Although not peer-reviewed, conference proceedings and government documents were also considered for inclusion, but other grey literature sources were not. Only English language

studies were accessed. However, English language abstracts were assessed, where available, for foreign language publications. Initial screening of all identified studies was undertaken and textbook chapters, letters, review articles, non-equine studies and those not pertaining to PPID were excluded at this stage. The abstracts of remaining studies were then screened. Studies where PPID pathophysiology, pergolide pharmacology, diagnostic test accuracy or other endocrinological parameters were the outcome measure, as well as those which did not contain information relevant to the review question were excluded. If abstracts reported findings from the same study as a full publication, only the full publication was assessed to avoid duplication. Eligibility assessment was carried out independently in a standardised, unblinded manner by the authors.

Review methodology

Study design, methodology and information reported in each included study was evaluated using appropriate STROBE checklists (checklists for cohort, case-control and cross-sectional studies combined or conference abstracts (Anon, 2007a) (Appendices 2 and 3). Quality assessment scoring was not undertaken. Studies were not excluded on quality grounds; however, results of lower quality studies are presented with interpretation of their limitations.

Standardised forms adapted from critically appraised topic (CAT) data collection forms were used to extract relevant data from included publications. Data extraction was undertaken individually by the authors and subsequently cross-checked by the other authors to ensure agreement was reached. No attempt was made to obtain missing data and investigators were not contacted to confirm accuracy of included information.

Data and relevant results extracted from each included publication are presented in summary tables (Appendices 4 and 5). Due to variations in study design, study populations and outcome measures between included studies, meta-analysis was not undertaken. Therefore, the results of qualitative evidence synthesis are presented in this review. However, overall prevalence estimates were calculated for clinical and endocrinological improvement in pergolide treated PPID cases, where numerator and denominator data were reported in included studies. Where 95% confidence intervals (CI) were not provided in the publication, these were calculated by the authors where published information permitted.

Table 1: Summary of clinical and endocrinological response to treatment with pergolide in horses diagnosed with PPID, in a systematic review of published literature.

Study	Study type	Number treated with pergolide	Pergolide dose at start of treatment (pergolide formulation where reported)	Active comparison treatment	Untreated control group	ACTH reference interval or cut-off value used to define PPID cases (test time of year where reported)	Outcome measure	Duration of pergolide treatment	Main results (95% confidence intervals [CI] calculated where data allowed)	Side effects
Aleman et al., (2006)	Case control study	n=3	1 mg/day PO (pergolide mesylate tablet; Permax, Eli Lilly)	None	n=12	2-10 pmol/L (4.5-45.5 pg/mL; recruited during winter and spring)	Clinical signs, ACTH and muscle biopsy	3 months	Improved body condition score, physical activity and shift in muscle fibre population in all 3 pergolide treated PPID cases ACTH did not return to within reference interval in the 3 pergolide treated cases	Not reported
Anon (2011a)	Uncontrolled, non-randomised field trial	n=122 n=113 available for follow-	0.002 mg/kg/day PO (pergolide mesylate tablet;	None	None	≤50 pg/mL (pre-treatment Nov – Jan, post	Clinical signs and ACTH or ODST	90 and 180 days (3 and 6 months)	76% (n=86/113; CI 68.2-84.0%) treatment success after 180 days treatment	33% transient inappetence; <10% of cases: lethargy, colic,

		up at days 90 and 180	Prascend, Boehringer Ingelheim)			treatment Jan – March and April – June)			58% (n=66/113; CI 49.3-67.5%) had normal endocrine results at day 90	diarrhoea, lameness, and weight loss
Beech et al., (2002)	Uncontrolled, non-randomised field trial (of Vitex agnus castus)	n=9	0.002-0.006 mg/kg/day PO	Vitex agnus castus n=14 Of which, n=9 deteriorated and started pergolide treatment	None	≤35 pg/mL	Clinical signs, ACTH and ODST	Seven horses for 3 months, 2 for >4 months	89% improved clinically (n=8/9; CI 68.4-100%) ACTH decreased by ≥50% in 66.7% (n=4/6; CI 28.9-100%)	Lethargy reported in one pergolide treated horse
Beech et al., (2009)	Non-randomised controlled trial	n=8	0.5 mg/day PO for ponies 3 mg/day PO for horses (formulation not stated; produced by compounding pharmacy, Wickliffe Pharmaceuticals)	None	n=7	9-35 pg/mL (multiple tests performed Feb-Oct)	ACTH	7 months	Pergolide treated cases had lower ACTH values compared to untreated controls	Not reported
Christen et al., (2018)	Randomised placebo-controlled trial	n=10	0.002 mg/kg/day PO (pergolide mesylate tablet; Prascend,	None	n=9 placebo treated	<35 pg/mL (pre-treatment July, post-treatment Jan)	ACTH	3 months	ACTH values significantly lower in pergolide group ($p<0.01$): Median ACTH in pergolide group =	Not reported

			Boehringer Ingelheim)						25.9 pg/mL (range 7.4-50.1 pg/mL) Median ACTH in placebo group = 73.2 pg/mL (range 37.8-479 pg/mL)	
Donaldson et al., (2002)	Case series	n=20	0.0017-0.055 mg/kg/day PO (median dose0.003 mg/kg/day)	Cyproheptadine n=7 0.25 mg/kg/day PO	None	9-35 pg/mL (recruited Jun-Nov)	Clinical signs and ACTH	1-10 months; median 2 months	Clinical signs improved: 85% pergolide (n=17/20; CI 69.4-100%) 28% cyproheptadine (n=2/7; CI 0-62.0%) ACTH normalised in 60% of pergolide group (n=12/20; CI 38.5-81.5%) and none of cyproheptadine group	Not reported
Dunkel et al., (2014)	Case series	n=5	0.0007-0.008 mg/kg/day PO (median starting dose 0.0038 mg/kg/day PO)	None	None	Variable reference intervals used for individual cases	Clinical signs and ACTH	n=1 for 1 month, n=1 for 2 months, n=1 for 12 months, n=1 for 18 months and n=1 for 5 years	Clinical improvement in 80% (n=4/5; CI 45.0-100%) ACTH follow-up reported in three cases: ACTH levels improved in one horse, improved at first follow- up then	Depression, anorexia and heart murmur observed in one horse - not observed subsequently on reduced dose.

									subsequently increased in one horse and remained elevated in one horse	
Froin et al., (1998)	Case series	n=5	0.0017 mg/kg/day PO	None	None	Not reported	Clinical signs, ACTH, ODST, cortisol	12 months	100% clinical improvement (n=5/5; CI 56.6-100%) Improvement in ACTH reported in one horse ODST normal after treatment in three cases	Not reported
Gehlen et al., (2014)	Case control study	n=13	0.5-2mg/day PO (pergolide mesylate tablet; Prascend, Boehringer Ingelheim)	None	No treatment n=25	≥50 pg/mL (pre-treatment in Jan)	ACTH	Minimum 3 months	Treatment did not prevent laminitis occurring Trend towards lower ACTH values in treated horses but not statistically significant	Not reported
Horn et al., (2019)	Retrospective cohort study	n=218, of which 143 had clinical follow-up data	0.0005-0.0125 mg/kg/day PO (median 0.002 mg/kg/day); dose increased in 61 cases (for which median dose remained	None	247 cases had medication data recorded, therefore presumed to be no	Variable cut-off values adjusted by season and location: autumn cut-off (used for 38.5% of cases at	Clinical signs and ACTH	Not reported. Median duration of follow-up across all cases 11 months	64.3% clinical improvement (n=92/143; CI 56.5-72.2%) ACTH returned to within reference intervals in 44.4% (n=52/117; CI 35.4-53.4%).	Not reported

			0.002 mg/kg/day) (of the 119 cases where reported, 45.6% commenced treatment on a liquid pergolide formulation and 54.6% on a tablet form)		treatment n=29	diagnosis) range 77.4 – 101 pg/mL and non-autumn cut-off (used for 61.5% of cases at diagnosis) range 29.7 – 67 pg/mL		(range 0 – 85 months)	No untreated cases had normal ACTH concentrations at repeat testing.	
Innerå et al., (2013)	Uncontrolled, non-randomised field trial	n=8	0.002 mg/kg/day PO, increased to 0.004 mg/kg/day PO after 3 months in all cases (pergolide mesylate tablet; Prascend, Boehringer Ingelheim)	None	None	Not measured	Clinical signs, hypertrichosis score, hair follicle stages on skin biopsy	6 months	Clinical improvement in hypertrichosis: shedding 100% (CI 67.6-100%), decrease in anagen phase hair follicles 50% (n=4/8; CI 15.4-84.6%) had normal ODST results after 6 months of treatment	Not reported
Love (1993)	Case series	n=1 after treatment failure with cyproheptadine	3mg/day PO, decreasing to 1 mg/day after 6 weeks and 1mg every	Cyproheptadine n=4 0.6-1.2mg/kg$^{0.75}$	n=1	Not measured	Clinical response	>12 weeks	Improvement in clinical signs in pergolide treated pony. 25% (n=1/4; CI 0.0-67.4%)	Transient anorexia in pergolide treated pony when on high

			other day thereafter						improved on cyproheptadine. No change in control horse.	dose (3mg daily)
McFarlane et al., (2017)	Uncontrolled, non-randomised clinical trial	n=6	1 mg/day PO, increasing to 2 mg/day after 2 months (pergolide mesylate tablet; Prascend, Boehringer Ingelheim)	None	None	Basal ACTH and ACTH measured 30 min after TRH stimulation	ACTH	6 months	Basal ACTH significantly lower at 4, 5 and 6 month time points but not 30 min post TRH	None reported
Orth et al., (1982)	Case series	n=1	5 mg dose single dose PO (pergolide mesylate 5mg capsule, Eli Lilly)	Bromocriptine n=1, 100mg dose (oral and subcutaneous)	None	Not reported	POMC peptides including ACTH	48 h	Decreased ACTH following single dose	None reported in pergolide treated horse. Bromocriptine had to be given S/C with reactions under skin as no availability orally
Pease et al., (2011)	Case control study	n=6	1 mg/day PO, increased to 2 mg/day after 3 months for 3/6 cases (pergolide mesylate	None	n=2	Not measured	Pituitary size on computed tomography	6 months	Pituitary length increased, height and width unchanged compared to pre-treatment. Clinical improvement in all	Not reported

			tablets; Lilly Celance, Eli Lilly or pergolide mesylate tablets, Elanco Animal Health)						6 pergolide treated cases after 3 months (n=6/6; CI 61.0-100%) ODST normalised in 83% (n=5/6; CI 53.5-100%) cases after 3 or 6 months of pergolide	
Perkins et al., (2002)	Retrospective cohort study	n=10	0.75-1.0 mg/day (~0.0017 mg/kg/day) PO (n=7); 1.5-2mg/day (n=1) or 0.38-0.5mg/day (n=2).	Cyproheptadine n=32 225-250 mg/day (either 0.5 mg/kg once daily or 0.25 mg/kg every 12 h; n=5) or 450-500 mg/day (n=27)	None	9-35 pg/mL (recruited Apr-Dec)	ACTH and veterinary surgeon survey	1-15 months; median 2 months (including 2 re-checks)	90% (n=9/10; CI 71.4-100%) pergolide treated and 81% (n=26/32; CI 67.7-94.8%) cyproheptadine-treated horses showed improvement in at least 1 clinical sign ACTH improved in ~ 60% horses on either treatment; correlated with improvement in hypertrichosis in pergolide group only	Not reported
Peters et al., (1995)	Case series	n=9	0.0017 mg/kg/day PO BID	None	None	Not measured	Clinical signs	1-23 months,	89% (n=8/9; CI 68.4-100%) clinical improvement	No adverse effects

			(pergolide mesylate tablet; Permax, Eli Lilly)					mean 13.7 months	ODST showed improvement in 71.5% (n=5/7; CI 38.0-100%)	
Pongratz et al., (2010)	Retrospective cohort study	n=38	0.001-0.002 mg/kg/day PO (median dose 0.001 mg/kg/day PO) (pergolide mesylate tablet; Permax, Eli Lilly)	None	None	<50 pg/mL in horses and <30 pg/mL in ponies	Owner questionnaire on clinical signs	1-38 months, mean 13.8 months	Clinical improvement in 88.6% (n=31/35; CI 78.0-99.1%) 90% (n=34/38; CI 79.7-99.2%) owners satisfied with treatment	n=11 transient mild adverse effects, (anorexia, lethargy, diarrhoea)
Rendle et al., (2013)	Retrospective cohort study	n=2,122	Variable	None	None	Seasonally adjusted reference intervals (values not reported)	ACTH	Not reported	54.8% (n=1,163/2,122; CI 52.7-57.0%) ACTH improvement; 28% (n=594/2,122; CI 26.1-30.0%) returned to within reference intervals. Duration of treatment was positively associated with treatment response (p=0.04)	Not reported

Rendle et al., (2018)	Retrospective cohort study	n=19	Not reported (pergolide paste; BOVA UK)	None	None	>50 pg/mL (July) or >100 pg/mL (August – October)	Clinical signs, ACTH and insulin	6 months	Unable to determine improvement in clinical signs from data presented. No significant improvement in insulin. 73.7% (n=14/19; CI 53.9-93.5%) improvement in ACTH to within reference interval.	Inappetence observed in two ponies, which resolved after dose reduction
Rohrbach et al., (2012)	Retrospective cohort study	n=10 n=5 pergolide plus cyproheptadine	Not reported	Cyproheptadine n=7	None	Not measured	Owner based telephone questionnaire on clinical signs	2 months	Positive response in 40% (n=4/10; CI 9.9-70.4%) pergolide; 29% (n=2/7; CI 0-62.0%) cyproheptadine, and 60% (n=3/5; CI 17.0-100%) receiving both drugs	Not reported
Schott et al., (2001)	Uncontrolled, non-randomised field trial	n=20	0.002 mg/kg/day PO	Cyproheptadine n=7 1.2 mg/kg/day PO	n=5	Not measured	Clinical signs	6-12 months	Clinical improvement with pergolide. A 'few' also with cyproheptadine. No improvement in untreated control group.	Decreased appetite seen in several pergolide treated horses in the first week, resolved with transient

									Normal ODST and/or TRH stimulation results in 35% of pergolide group (n=7/20)	reduction in dose
Schott et al., (2014)	Retrospective cohort study	n=30	0.002mg/kg/day PO, increased to 0.004 mg/kg/day where endocrine test results remained abnormal (pergolide mesylate tablet; Prascend, Boehringer Ingelheim)	None	None	<50 pg/mL	ACTH and clinical signs	5.5 years	100% of survivors (n=12/12; CI 75.8-100%) had clinical improvement Where tested, ACTH was normal (<50 pg/mL) in 71% (CI 45.1-96.6%) survivors and ODST results remained normal in 61% (CI 33.2-88.5%) of survivors	Not reported
Sgorbini et al., (2004)	Case series	n=2	0.5 mg/day PO, increased by 0.5 mg every 3 days to final dose of 3 mg once daily (pergolide mesylate; Nopar, Lilly)	None	None	≤35 pg/mL	ACTH and clinical signs	Not stated	Clinical signs 'resolved' and ACTH values improved in both cases	None reported

Spelta and Axon (2012)	Case series	n=7	0.001 mg/kg/day PO (pergolide mesylate tablet; Permax, Draxis Health or liquid formulation; Ranvet Pergolide, Ranvet)	None	n=4	Not measured	Clinical signs	2 months to 7 years	100% (n=7/7; CI 64.6-100%) reported to show improvement in clinical signs in pergolide group. No clear improvement in non-treated group.	Not reported
Walsh et al., (2009)	Case series	n=6	1 mg/day PO	None	None	9-35 pg/mL (cases only included if >70 pg/mL; recruited Dec-May)	ACTH	2 months to 2.5 years	Decrease in ACTH in 1 week, remained ~70 pg/mL	Not reported
Watson et al., (1998)	Case series	n=6	0.0018-0.0028 mg/kg PO	None	None	Not measured	Clinical signs	6-26 months	100% (n=6/6; CI 61.0-100%) reported to show improvement in clinical signs	No adverse events
Williams (1995)	Case control study	n=14 Received cyprohepadine and/or pergolide	Low dose: 1-2 mg/day High dose: 4-5 mg/day PO (pergolide mesylate tablets; Celance, Lilly)	Cyproheptadine	n=9	Not reported	Clinical signs, cortisol and TRH stimulation tests	Up to 3.5 years	Improvement in all horses on high dose pergolide, also 'encouraging' results on low dose. n=4/9 untreated cases euthanased. Cyproheptadine showed only	Not reported

									temporary improvement.	
Overall		Total horses treated with pergolide and available for follow-up n=2,649	Most frequently reported median dose 0.002 mg/kg/day		Total controls n=105				Where proportions were reported or calculated, clinical improvement was observed in 77.0% (n=328/426; CI 73.0-81.0%) of cases	
									Where proportions were reported or calculated, ACTH values improved or returned to normal in 54.9% (n=1,338/2,437; CI 52.9-56.95%) of cases	

CI = 95% Confidence interval; ACTH = Adrenocorticotrophic hormone; ODST = Overnight dexamethasone suppression test; PO = per os; POMC = Pro-opiomelanocortin; TRH = Thyrotropin-releasing hormone

Results

A total of 833 records were identified via the combined electronic database searches, with an additional three records obtained from other sources. Following removal of duplicate records, 612 records were screened for relevance to the review question. Following title and abstract screening, a total of 41 publications that investigated treatment with pergolide were evaluated in full. Thirteen publications did not meet inclusion criteria and were subsequently excluded, resulting in 28 studies included in the review (Figure 1). The included publications comprised descriptive case series (n=10), cohort studies (n=7), uncontrolled non-randomised trials (n=5), case control studies (n=4), a randomised placebo-controlled trial (n=1) and a non-randomised controlled trial (n=1).

Study populations included were predominantly from university referral hospitals, university research herds or other referral hospitals (n=12). Five studies had field-based populations selected from first opinion practice and several had mixed populations including referral hospitals, research herds and/or private practice (n=5). One study was based at an equine retirement sanctuary and five studies did not provide sufficient details to ascertain the population setting (Appendices 4 and 5).

Case inclusion criteria varied between studies; the majority of PPID cases were diagnosed via a combination of clinical signs and various diagnostic tests including basal ACTH concentration and/or other diagnostic tests such as the overnight dexamethasone suppression test (ODST) and thyrotropin-releasing hormone (TRH) stimulation test (n=21). Other case inclusion criteria were elevated basal ACTH concentration alone (n=3), clinical signs alone (n=1), ODST alone (n=1), and TRH stimulation test alone (n=1). Diagnostic criteria were not reported in one study (Appendix 5). A total of 15 studies utilised basal ACTH concentration for PPID diagnosis in all or some of the included cases. One of these studies reported utilising contemporaneous seasonally adjusted reference intervals (Rendle et al., 2013) and a case series used values above seasonally adjusted reference intervals in three of the four PPID cases diagnosed using basal ACTH (Dunkel et al., 2014), while a further study (Rendle et al., 2018) utilised clinical decision limits recommended by the Equine Endocrinology Group (EEG, 2019). One further study utilised seasonally adjusted reference intervals that varied dependent on geographical location; however, these reference intervals were not contemporaneous for a considerable proportion of the study period (Horn et al., 2019). Two studies completed case enrolment and follow-up testing

avoiding the autumn seasonal rise in ACTH (July – October in the Northern hemisphere; Aleman et al., 2006; Anon, 2011a).

Across all included publications, a total of 2,740 horses and ponies were treated with pergolide with 2,649 of these cases including some form of follow-up observation. The most frequently reported median starting dose of pergolide was 0.002 mg/kg/day (range 0.001 – 0.0125 mg/kg/day; Table 1). The majority of studies assessed treatment with pergolide alone (n=15), with no comparison group or control group (Peters et al., 1995; Froin et al., 1998; Watson et al., 1998; Sgorbini et al., 2004; Aleman et al., 2006; Walsh et al., 2009; Pongratz et al., 2010; Anon, 2011a; Pease et al., 2011; Innerå et al., 2013; Rendle et al., 2013; Dunkel et al., 2014; Schott et al., 2014; McFarlane et al., 2017; Rendle et al., 2018). The remainder assessed pergolide treatment in comparison with no treatment (n=4; Beech et al., 2009; Spelta and Axon, 2012; Gehlen et al., 2014; Horn et al., 2019), a placebo treatment (n=1; Christen et al., 2018) or in comparison to an active control group receiving an alternative unauthorised treatment including cyproheptidine (n=6; Love, 1993; Williams, 1995; Schott et al., 2001; Donaldson et al., 2002; Perkins et al., 2002; Rohrbach et al., 2012;) bromocriptine (n=1; Orth et al., 1982) or *Vitex agnus castus* (n=1; Beech et al., 2002). Three of these studies also included an untreated control group (Love, 1993; Williams, 1995; Schott et al., 2001; Table 1).

Clinical response

Reported improvement in at least one clinical sign following commencement of pergolide treatment ranged from 40-100% (Table 1), with a high proportion of cases (≥76%) showing clinical improvement in the majority of studies (Peters et al., 1995; Froin et al., 1998; Watson et al., 1998; Beech et al., 2002; Donaldson et al., 2002; Perkins et al., 2002; Aleman et al., 2006; Anon, 2011a; Spelta and Axon, 2012; Innerå et al., 2013; Dunkel et al., 2014; Schott et al., 2014). Evaluation of clinical response varied between studies with no standardised method used. Evaluation was most frequently based on subjective improvement in clinical signs, which was predominantly assessed via veterinary clinical examination (Williams, 1995; Schott et al., 2001; Beech et al., 2002; Perkins et al., 2002; Anon, 2011a; Pease et al., 2011; Innerå et al., 2013; Schott et al., 2014; Rendle et al., 2018; Horn et al., 2019), however owner assessment was also utilised (Peters et al., 1995; Donaldson et al., 2002; Pongratz et al., 2010; Rohrbach et al., 2012). A case series included both veterinary and owner-reported assessment of clinical improvement (Spelta and Axon,

2012), while the method of evaluation was either not reported or was unclear in five further studies that provided information on clinical response to pergolide treatment (Love, 1993; Froin et al., 1998; Watson et al., 1998; Sgorbini et al., 2004; Dunkel et al., 2014).

Two clinical trials used objective measures (Anon, 2011a; Innerå et al., 2013) providing some of the highest quality evidence regarding clinical improvement. One study was associated with licensing of pergolide tablets for use in horses (Prascend, Boehringer Ingelheim), where 76.1% of 113 enrolled horses assessed over a 6-month study period were considered treatment successes based on objective clinical assessment scores and/or endocrinological improvement (defined as either normalisation of ODST results or a post-treatment reduction in basal ACTH of ≥50% from pre-treatment values) (Anon, 2011a). The other study showed objective improvement in coat quality with a reduction in anagen phase hair follicles following 6 months of treatment with pergolide (Innerå et al., 2013).

Relative improvement in specific clinical signs following pergolide treatment could be determined in five studies. Improvement in hypertrichosis ranged from 30-100%, abnormal fat distribution 0-33%, hyperhidrosis 15-45%, lethargy/poor performance 20-47%, muscle wastage 21-46% and laminitis 32-75%, of cases treated with pergolide (Beech et al., 2002; Donaldson et al., 2002; Perkins et al., 2002; Pongratz et al., 2010; Anon, 2011a). High proportions of pergolide treated animals showing improvement in clinical signs were reported in both referral/hospital and field-based studies although more variation was seen within the referral/hospital populations (Figure 2). Clinical improvement was also observed over prolonged time periods, with eleven studies including follow-up periods of ≥12 months (Peters et al., 1995; Williams, 1995; Froin et al., 1998; Watson et al., 1998; Schott et al., 2001; Perkins et al., 2002; Walsh et al., 2009; Pongratz et al., 2010; Horn et al., 2019), including some horses followed for up to 5.5 years (Schott et al., 2014) and one horse was followed for 7 years (Spelta and Axon, 2012).

Only a small number of studies compared pergolide to other unauthorised medical treatments. When compared to the serotonin agonist cyproheptadine, pergolide was reported to be superior at alleviating clinical signs in all studies, with the majority reporting only 25-29% of cases improved clinically when treated with cyproheptadine (Love, 1993; Schott et al., 2001; Donaldson et al., 2002; Rohrbach et al., 2012). The exception was one study which reported that 81% of cases treated with cyproheptadine showed improvement

in at least one clinical sign, however this was still inferior to the proportion reported in pergolide treated cases (90%; Perkins et al., 2002). The herbal supplement *Vitex agnus castus* was also investigated in a single uncontrolled field study (Beech et al., 2002). Clinical deterioration was observed in all but one of 14 PPID cases treated with *Vitex agnus castus* and nine of these animals were subsequently treated with pergolide (Beech et al., 2002).

Plasma ACTH concentrations

The proportion of PPID cases demonstrating improvement in plasma ACTH concentrations following pergolide treatment ranged from 20-74% (Table 1). Laboratory reference intervals used for the measurement of ACTH varied between studies. The majority of included studies used either ≤35 pg/mL or ≤50 pg/mL as the upper limit of the reference interval when reporting response to treatment, dependent on the type of assay used (Table 1). Normalisation of plasma ACTH concentrations to within these non-seasonally adjusted reference intervals occurred in 58-71% of cases (Donaldson et al., 2002; Anon, 2011a; Schott et al., 2014), while a decrease in plasma ACTH concentrations was reported in 20-54.8% of cases (Froin et al., 1998; Beech et al., 2002; Rendle et al., 2013; Dunkel et al., 2014).

One retrospective study reportedly used seasonally adjusted reference intervals when measuring improvement in ACTH concentration following pergolide treatment, in which 28% of cases returned to within these intervals (Rendle et al., 2013). The same study reported a ≥75% reduction in basal plasma ACTH concentrations in 54.8% of 2,122 cases, and those with a higher initial plasma ACTH concentration were more likely to improve. Another retrospective study using seasonally and geographically adjusted cut-off values reported normalisation of plasma ACTH concentrations in 44.4% of cases overall, across multiple repeat tests over a prolonged period (Horn et al., 2019). One study reported that ACTH concentration returned to within reference intervals for 74% of cases following pergolide treatment; however only seasonally adjusted clinical decision limits for initial diagnosis and not reference intervals used for follow-up testing were reported (Rendle et al., 2018).

Two studies reported that ACTH concentration did not significantly decrease in pergolide treated cases compared to non-treated PPID controls (Gehlen et al., 2014) or pre-treatment values (McFarlane et al., 2017) over ≥3 months. Conversely, one placebo-controlled trial did report significantly lower plasma ACTH concentrations in pergolide treated cases over a

similar time period (Christen et al., 2018). There was some evidence to suggest that a prolonged time period or requirement for an increase in dose (from the median starting dose of 0.002 mg/kg/day) may be needed to achieve a satisfactory endocrine response (Williams, 1995; Spelta and Axon, 2012; Rendle et al., 2013; Schott et al., 2014; McFarlane et al., 2017).

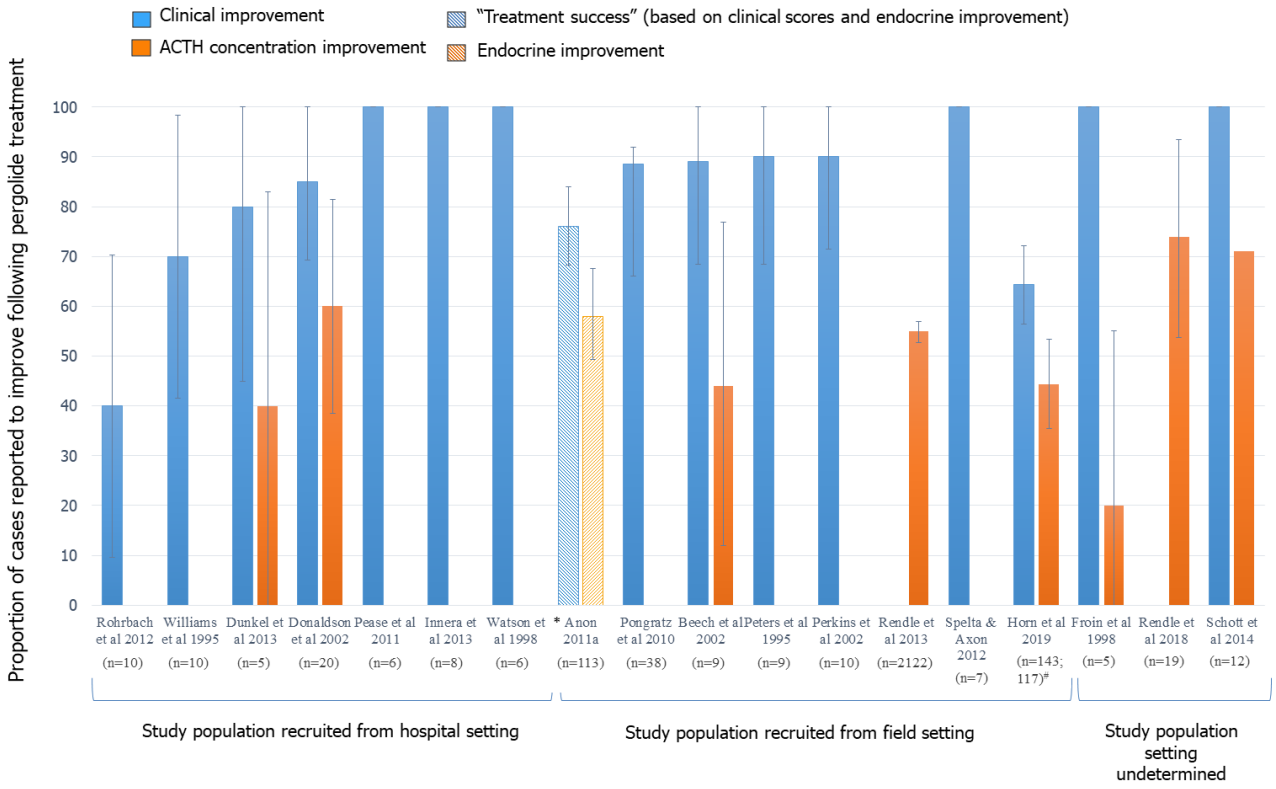


Figure 2: Reported proportion(s) of pituitary par intermedia dysfunction (PPID) cases treated with pergolide that showed improvement in clinical signs and/or endocrine levels in a systematic review of published literature. Error bars represent 95% confidence intervals, calculated by the authors where published information permitted. *Clinical improvement and adrenocorticotrophic hormone (ACTH) plasma concentrations could not be determined separately from the data provided. #In Horn et al., 2019, sample size differs for clinical (n=143) and ACTH concentration (n=117) improvement. Additionally, the study population was selected from both a hospital and a field setting.

Side effects and adverse events

Side effects attributed to pergolide administration were recorded in seven studies with transient inappetence most frequently reported (Love, 1993; Muñoz et al., 1996; Schott et al., 2001; Pongratz et al., 2010; Dunkel et al., 2014; Schott et al., 2014; Rendle et al., 2018; Table 1). The most robust study reporting the prevalence of side-effects found 33% of horses developed transient inappetence, which responded well to a short-term dose reduction (Anon, 2011a). Another study reported that 29% of pergolide treated cases developed mild side-effects including anorexia, lethargy and diarrhoea, which were generally self-limiting (Pongratz et al., 2010). Less than 10% of horses showed adverse events such as colic, lameness and laminitis (Anon, 2011a). Adverse events were reported whether related to the treatment under investigation or not, therefore it is not possible to determine whether these were directly associated with pergolide treatment (Anon, 2011a).

Discussion

This is the first paper to systematically review published evidence regarding the use of pergolide for the management of clinical signs and endocrine parameters associated with PPID.

A large proportion of the available evidence regarding the treatment of PPID with pergolide has come from descriptive case series or small hospital-based populations. These studies had varying case selection criteria, diagnostic protocols, pergolide doses, treatment durations and outcome measures. It is also likely that the product used was inconsistent. Early studies conducted before the current authorised equine formulation of pergolide mesylate used pergolide products authorised for human use, while others may have used compounded pergolide, which has been reported to differ in potency (Davis et al., 2009; Shank, 2009; Stanley and Knych, 2010). Additionally, one recent study evaluated an unauthorised pergolide paste formulation (Rendle et al., 2018). Despite these noted variations between studies, pergolide was consistently reported to provide overall improvement in clinical parameters in over 75% of reported cases. This high proportion of cases showing overall clinical improvement was observed in a large multicentre study performed as part of the licensing requirement for equine use (Prascend, Boehringer Ingelheim; Anon, 2011a), and in both field and hospital-based studies (Figure 2), with the exception of two retrospective cohort studies that had a high level of loss to follow-up

(Rohrbach et al., 2012; Horn et al., 2019). Improvement in individual clinical signs was more varied; this is likely to be due to the differences in treatment duration and selection criteria. Determining the efficacy of pergolide for treating specific clinical signs with any degree of certainty was not possible due to variability in reporting. Clinical signs of PPID are diverse (Schott, 2002) and the interrelationship between them is unclear (McFarlane et al., 2006; McFarlane, 2011). Further evidence is required regarding the effect of pergolide treatment on specific clinical signs, particularly laminitis due to its high prevalence and welfare impact (Herthel and Hood, 1999; Mellor et al., 2001; Karikoski et al., 2011).

Due to the number of confounding factors and lack of control groups, it is difficult to attribute the side effects or adverse events observed to pergolide treatment specifically. However, transient inappetence was reported in a relatively high proportion of cases, which resolved after a reduction in the dose administered (Anon, 2011a). It may, therefore, be beneficial to introduce pergolide gradually, especially when a higher dose is required. There is insufficient evidence regarding possible long-term side-effects of pergolide, however the studies where cases were followed for a prolonged period (>5 years) did not report any adverse effects (Spelta and Axon, 2012; Dunkel et al., 2014; Schott et al., 2014; Horn et al., 2019).

The life expectancy of horses in the UK is increasing (Ireland et al., 2011a; Welsh et al., 2016) and with efficient diagnostic testing readily available, cases of PPID are likely to be diagnosed and treated earlier. Median life expectancy following PPID diagnosis has been reported to be 9.8 years (Welsh et al., 2016), therefore in practice, pergolide treatment is likely to be undertaken for a number of years. There is some evidence to suggest this may have a protective effect against further neurodegeneration caused by oxidative stress (Gille et al., 2002). Pergolide treatment has been associated with increased odds of survival, supporting its use to improve prognosis in PPID cases (Horn et al., 2019). However, further evidence is needed regarding the benefits of early treatment and the efficacy of pergolide in the long term.

Plasma ACTH concentrations offer an objective measure of improvement in response to treatment, however, variation in the diagnostic assays, laboratory and sampling protocols as well as reference intervals used, made comparison between studies challenging. In the UK, measurement of basal ACTH using seasonally adjusted reference intervals is the primary

endocrine test used to diagnose PPID and monitor response to treatment. This test is reported to provide a high level of sensitivity and specificity, accounting for seasonal variations in basal plasma ACTH concentrations (McGowan et al., 2013b). As seasonally adjusted reference intervals were established fairly recently (Copas and Durham, 2012), the majority of included studies used non-seasonally adjusted reference intervals when measuring response to treatment. Therefore, the likelihood of non-differential misclassification bias is increased (Jurek et al., 2005), and this could lead to cases being falsely classified as having 'abnormal' plasma ACTH concentrations during the autumn seasonal rise or 'normal' concentrations in non-autumn months. However, despite this, such studies are still useful for determining improvement in plasma ACTH concentrations. One clinical trial reported significantly lower ACTH values in pergolide treated cases compared to placebo treated controls (Christen et al., 2018). However, in general, reduction in plasma ACTH concentrations was much more varied than overall improvement in clinical signs, and the two did not always correspond (Froin et al., 1998; Donaldson et al., 2002; Anon 2011a; Dunkel et al., 2014; Schott et al., 2014). This is not unexpected; the clinical signs of PPID are multifactorial and ACTH is one of many POMC-derived peptides which increase as a result of PPID (Heinrichs et al., 1990; McFarlane, 2011). ACTH is utilised as a biomarker because assays to measure it are widely available (Wilson et al., 1982) and sampling is convenient (Rendle et al., 2015a). However, plasma ACTH concentrations have been reported to be affected by external factors such as stress and pain (Fazio et al., 2008; Towns et al., 2010) as well as exercise (Alexander et al., 1991; Nagata et al., 1999) and ingestion of feed (Diez de Castro et al., 2014). Further evidence is required to fully interpret the clinical picture as a whole and the relationship between endocrine parameters and clinical signs.

Where study population was able to be adequately determined, all included studies had potential biases (Appendices 4 and 5). Small convenience samples recruited via hospital or referral practices are likely to be biased towards cases in the advanced stages of disease or with concurrent disease(s), so results are not likely to be generalisable to the general population. Samples from first opinion practice have the potential to be more representative of the target population, however, the number of treated cases in many studies was small ($n \leq 30$), reducing study power, and convenience sampling was still used. Two field-based studies had larger study populations (Pongratz et al., 2010; Anon, 2011a), however due to non-random selection and the necessity for voluntary recruitment of cases, there is the

possibility of selection bias; systematic differences between owners and cases which participated compared to those that did not. Rendle et al. (2013a) had the largest study population, however this study used data from a retrospective review of laboratory data, which has inherent limitations, such as the possibility of incomplete or incorrect records. Additionally, it is likely that the accuracy of signalment and clinical data provided on laboratory submission forms could not be verified (Rendle et al., 2013). Furthermore, many studies did not use an objective measure for clinical signs or a control group (Peters et al., 1995; Froin et al., 1998; Watson et al., 1998; Beech et al., 2002; Donaldson et al., 2002; Perkins et al., 2002; Pongratz et al., 2010; Rohrbach et al., 2012; Dunkel et al., 2014; Schott et al., 2014; Rendle et al., 2018), meaning assessment bias is probable. Veterinary surgeons and owners may expect to see improvement as a result of treatment and overestimate efficacy, thus resulting in bias towards a positive effect. However, the results from these studies were comparable to studies that did use objective assessment (Aleman et al., 2006; Anon, 2011a; Innerå et al., 2013) or a control group (Love, 1993; Williams, 1995; Schott et al., 2001; Spelta and Axon, 2012).

The lack of adequate control groups also means other potentially confounding factors, such as changes in management post-diagnosis cannot be controlled for. In their small case series, Spelta and Axon (2012) detailed the standardised management advice that was provided to owners, including dietary and preventive health care recommendations. Since many of these management changes could have an impact on clinical signs associated with PPID, and therefore the outcome of clinical response to treatment, they may be considered as co-interventions. Where co-interventions are not balanced across the study population, there is potential for performance bias, and none of the studies reviewed reported making any considerations for co-interventions in the evaluation of response to treatment. It is therefore possible that some clinical improvements attributed to a positive response to pergolide treatment could actually reflect a response to improved husbandry and general health care.

While studies including client-owned animals are likely to be more representative of the general population of PPID cases, compared to experimental studies, or those undertaken within research herds, there is a necessary reliance on owners correctly and successfully administering the prescribed dose of pergolide. None of the publications where the study population included client-owned animals reported making any attempts to evaluate owner

compliance with pergolide treatment recommendations. Non-adherence to, or departures from, the intended pergolide treatment represent another important potential source of bias.

Overall, the available evidence demonstrates improvement in clinical and endocrine parameters in response to pergolide treatment. However, the evidence base is limited with outcomes observed frequently being poorly defined. Ideally, a randomised, placebo controlled clinical trial would be conducted to appropriately evaluate pergolide as an effective treatment for PPID. However, it may not be considered ethical to withhold an authorised treatment which has been shown to improve animal welfare (Fenwick et al., 2009). Instead, further research should concentrate on the interrelationship between endocrine parameters and clinical signs to improve interpretation of the clinical signs, the effect of early intervention and long-term administration as well as the efficacy of pergolide for treating specific clinical signs such as laminitis.

Conclusion

Although the evidence available regarding the efficacy of pergolide treatment is varied and with potential biases, the current evidence indicates that treatment with pergolide is effective at improving clinical signs of PPID in the majority of cases. However, the reported efficacy for improving plasma ACTH concentrations was more varied. Further evidence is required to better inform clinical decision making.

Declaration

This chapter was included with permission from the co-authors of the published version.

C. McGowan contributed to independent review of included studies and preparation and approval of the manuscript. J. Ireland contributed to independent review of included studies, study design and preparation and approval of the manuscript.

Appendices for Chapter 4

Appendix 1: Search strategy and results

Databases searched and dates covered	NCBI PubMed (1950 – Present) Clarivate Analytics Web of Science (1898 – Present) CAB Direct (1960 – Present) Scopus (1823 – Present) International Veterinary Information Service (IVIS) database (1997 – Present) Further relevant records were identified by the authors via the bibliographies and reference lists of retrieved publications and published conference proceedings.
Search terms	(Equine Cushing* OR Pituitary pars intermedia dysfunction OR PPID OR Hyperadrenocorticism OR Pituitary adenoma) AND (horse* OR pony OR ponies OR equine OR equid*) AND (treatment* OR therapy OR therapeutic* OR medication* OR management)
Dates searches performed	23/04/2019, 06/08/2019, 15/10/2019 and 31/07/2020

Database	**Number of results**	**Excluded – non-English language publication**	**Excluded – non-systematic review article, review in conference proceedings or letter**	**Excluded – pharmacokinetic/*in vitro*/*in vivo* experimental study**	**Excluded – did not answer review questions**	**Total relevant papers**
NCBI PubMed	156	2	30	5	87	32
Clarivate Analytics Web of Science	154	2	41	6	65	40
CAB Direct	245	12	122	3	88	20
Scopus	249	7	64	4	132	42
International Veterinary Information Service (IVIS) database	29	4	7	1	13	4
Other sources	3					
Total relevant papers when duplicates removed, following title and abstract screening						**28**

Appendix 2: Study design, methodology and information reported in included studies based on STROBE checklist for cohort, case-control and cross-sectional studies (combined)

Reference	STROBE item from checklist for cohort, case-control, and cross-sectional studies (combined)																				
	Title and abstract	Introduction		Methods						Results							Discussion				Other information
	Title and abstract	Background	Objectives	Study design	Setting	Participants	Variables	Data sources/ measurement	Bias	Study size	Quantiative variables	Statistical methods	Participants	Descriptive data	Outcome data	Main results	Other analyses	Key results	Limitations	Interpretation	Generalisability	Funding
Aleman et al., 2006	?	+	+	+	?	?	+	+	?	-	+	+	+	+	+	+	?	+	-	?	-	-
Anon 2011	?	+	+	+	+	?	+	+	-	-	+	?	+	+	+	+	#	#	#	#	#	?
Beech et al., 2009	?	?	?	+	+	?	?	+	?	-	?	+	+	+	?	?	?	+	?	+	?	-
Christen et al., 2018	?	?	+	+	+	+	+	+	-	?	+	+	+	+	+	+	#	+	?	?	?	-
Donaldson et al., 2002	+	+	+	+	+	+	+	+	-	#	+	+	+	+	+	+	#	+	?	+	?	-
Dunkel et al., 2013	+	?	+	+	+	?	+	?	-	#	#	#	+	+	+	+	#	+	-	+	-	-
Gehlen et al., 2014	+	+	+	+	?	?	+	+	?	-	+	?	?	+	+	?	#	?	-	?	-	-
Horn et al., 2019	+	+	+	+	?	+	+	+	?	#	?	?	+	+	+	?	#	+	?	+	?	+
Innerå et al., 2013	+	+	+	+	+	?	+	+	?	-	+	+	+	+	+	+	#	+	+	+	?	+
Love 1993	-	+	?	+	+	?	?	+	-	#	?	#	+	+	+	+	#	+	-	+	?	-
McFarlane et al., 2017	+	+	+	+	?	+	+	+	+	-	+	+	+	+	+	+	#	+	?	+	?	?
Orth et al., 1982	?	+	+	+	?	?	+	+	-	#	+	#	+	+	+	+	#	?	-	?	?	?
Pease et al., 2011	+	+	+	+	+	?	+	+	?	-	+	?	+	+	+	+	+	+	?	+	?	-
Perkins et al., 2002	+	+	+	+	?	?	+	+	-	-	+	+	?	?	+	+	+	+	+	+	?	-
Pongratz et al., 2010	+	+	+	+	+	?	+	+	-	-	+	+	?	+	+	+	#	?	+	+	?	-
Rorhbach et al., 2012	+	+	+	+	+	+	?	+	-	-	+	?	?	+	+	+	?	+	-	+	-	+
Sgorbini et al., 2004	+	+	-	+	+	-	+	+	-	#	?	#	+	+	+	+	#	+	-	+	?	-
Spelta and Axon 2012	+	+	?	+	+	?	+	+	-	#	?	#	?	+	+	+	#	+	-	?	?	-
Walsh et al., 2008	+	+	+	+	+	+	+	+	-	#	+	+	+	+	+	+	#	+	?	+	+	+

Key:

- + Checklist item addressed
- ? Checklist item partially addressed/partial information reported
- - Checklist item not addressed/insufficient information reported
- # Checklist item not applicable

Appendix 3: Study design, methodology and information reported in included studies based on STROBE checklist for conference abstracts

Reference	STROBE item from checklist for conference abstracts										
					Methods				Results		
	Title	Authors	Study design	Objective	Setting	Participants	Variables	Statistical methods	Participants	Main results	Conclusions
Beech et al., 2002	?	+	?	+	?	+	+	#	+	+	+
Froin et al., 1998	?	+	?	+	?	?	+	#	+	?	+
Peters et al., 1995	?	+	?	+	+	+	+	#	+	?	+
Rendle et al., 2013	?	+	+	+	+	+	+	?	+	+	?
Rendle et al., 2018	-	+	+	+	?	?	?	-	+	?	+
Schott et al., 2001	-	+	?	+	+	+	+	?	+	?	+
Schott et al., 2014	?	+	?	-	+	?	?	#	+	?	+
Watson et al., 1998	?	+	?	+	?	?	+	#	+	?	+
Williams 1995	+	+	?	-	-	-	?	#	?	?	+

Key:

+	Checklist item addressed
?	Checklist item partially addressed/partial information reported
-	Checklist item not addressed/insufficient information reported
#	Checklist item not applicable

Appendix 4: Summary tables and data of included studies

A summary of extracted data for each included study, main limitations of the studies and conclusions are presented in the tables below.

ACTH = Adrenocorticotrophic hormone

ODST = Overnight dexamethasone suppression test

TRH = Thyrotropin-releasing hormone

Author, year: **Type of publication:**	Aleman et al., 2006 Journal publication
Study Design:	Case control study
Aim/Objective of the Study:	To compare muscle characteristics and haematological and serum biochemical parameters between PPID cases and normal horses.
Setting:	University referral hospital
Study Population:	Non-random selection –PPID cases from university research herd or presented to a university hospital; details on recruitment of horse owners/selection of horses not reported. Controls: n=16 normal horses • normal ODST or ACTH tested during winter and spring PPID cases: n=15 • Quarter Horse (n=5), Thoroughbred (n=4), Standardbred (n=2), Arab (n=2), Appaloosa (n=1) and Mustang (n=1) • diagnosed based on clinical signs and positive ODST or basal ACTH • 11 female, 4 male • age range 15 – 28 years (median 21 years) • 3 of these PPID cases treated with pergolide
Intervention Investigated:	*Control Group:* n=12 PPID cases not treated with pergolide due to financial constraints.
	Treatment Group: n=3 PPID cases treated with 1mg pergolide mesylate (Permax®) once daily for 3 months.
Outcome Measures:	Clinicopathological findings, clinical signs, electromyography, muscle biopsy
Main Findings:	*Haematology and biochemistry:* • no abnormalities of haematological and biochemical parameters in control group • mild leucocytosis with neutrophilia and lymphopenia in 1 PPID horse; hyperglycaemia in 3 PPID horses; increased sorbitol dehydrogenase and gamma glutamyl transferase in 1 PPID horse *Electromyography:*

	• EMG studies were not performed after treatment with pergolide mesylate *Muscle biopsy:* • PPID cases had mild non-specific myopathic alterations including myofibre size variation (n = 15), internal nuclei (n = 11), perimysial, endomysial, and sarcoplasmic fat accumulation (n = 7) • muscle wasting in PPID horses was the result of atrophy of types 2A and 2B muscle fibres and loss of type 2B myofibres *Pergolide treatment (n=3):* • treatment with pergolide improved body condition score and physical activity • no statistical differences in myopathic alterations of horses with PPID before and after pergolide treatment • muscle fibre type composition was significantly different following pergolide treatment: type 1 myofibres decreased and types 2A and 2B increased • ACTH did not normalise (reference interval 2 – 10 pmol/L) following pergolide
Interpretation of Results:	Sample selection likely to introduce bias. Baseline details of treated and untreated PPID cases unspecified. Some laboratory tests poorly described. Neutrophilia, lymphopenia, hyperglycaemia and increased liver enzymes occasionally seen in PPID cases. Muscle atrophy in PPID cases is caused by Type II muscle fibre myopathy – change in muscle fibre composition identified in all cases; however not clear if all PPID cases showed the same degree of myopathic changes. Very small sample size treated with pergolide. Treatment for PPID with pergolide resulted in clinical improvement (improved body condition score and physical activity) and showed beneficial effect on muscle fibre type composition.

Author, year: **Type of publication:**	Anon 2011a Freedom of Information Summary – NADA 141-331
Study Design:	Uncontrolled, non-randomised field trial
Aim/Objective of the Study:	To evaluate the effectiveness of pergolide mesylate in controlling the clinical signs associated with PPID in horses under field conditions.
Setting:	University referral hospital, research herds and private practice
Study Population:	122 equids diagnosed with PPID (age range 10 – 35 years; 59 male, 63 female, bodyweight 137-621 kg, and 16 breeds) at eight sites. Animals were scored (0-3) for hypertrichosis, hyperhidrosis, polyuria-polydipsia, abnormal fat distribution, and muscle wasting. Inclusion criteria were a hypertrichosis score >1 and either a plasma ACTH concentration >50 pg/ml or failure of endogenous

	cortisol concentration to suppress (<1.0 µg/dl) 19 hours after intramuscular dexamethasone administration (40 µg/kg), autumn seasonal rise was avoided
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=122 treated with oral pergolide mesylate (2 µg/kg, once daily) Treatment with oral pergolide mesylate (2 µg/kg once daily per os) was started within 7 days of initial evaluation. Animals were re-evaluated (physical exam and endocrine testing) after 90 (n=113) and 180 days (n=111) of treatment. When endocrine test results remained abnormal at 90 days (n=47), the pergolide dose was increased to 4 µg/kg orally once daily.
Outcome Measures:	Clinical signs and endocrine testing. Treatment success after 180 days was defined as either normalisation of ODST results (<1.0 ug/dl) or a decrease in plasma ACTH concentration by 50% (or to <50 pg/ml but with a reduction of at least 5 pg/ml) and improvement by a score of >1 in at least one clinical sign. Treatment was also considered successful when the sum of clinical scores decreased by >3, regardless of endocrine test results.
Main Findings:	76% (86/113) equids were treatment successes (two horses withdrawn by their owners between 90 and 180 days were categorised as treatment failures). The remaining nine animals died (n=8) or were euthanized (n=1) due to worsening of pre-existing conditions (laminitis and dental disorders) or colic. After 90 days of treatment, 58% (66/113) had normal endocrine test results and clinical improvement. Both median scores of clinical signs and mean concentrations of ACTH and cortisol (following dexamethasone administration) decreased during the study period. Transient inappetance was the most common adverse event observed in 40/122 (33%) equids, mostly during the initial 30 days of treatment. Other adverse events reported included lethargy, colic, diarrhoea, lameness, and weight loss in less than 10% of cases and it was unclear whether or not these events were related to use of the drug. There were ten reports of laminitis during the study: seven were recurrent episodes in animals with a previous history of laminitis and three were apparently new cases of laminitis.
Interpretation of Results:	Limited information regarding selection of study population, therefore not possible to assess risk of bias. Enrolment of all PPID cases during winter period however follow-up examinations and endocrine tests conducted January – March and April – June avoiding seasonal increases in reference intervals. This open label field efficacy study demonstrated that pergolide is effective in improving endocrine parameters and clinical signs of PPID in the majority of treated horses; however, not all clinical problems may be fully corrected (e.g. laminitis). All disease events occurring during trial reported as adverse reactions. Of the adverse events reported, only transient inappetance was noticeably higher than previous reports of disease frequency in aged horses.

Author, year: **Type of publication:**	Beech et al., 2002 Conference abstract
Study Design:	Uncontrolled, non-randomised field trial
Aim/Objective of the Study:	To evaluate the effects of Vitex agnus castus extract for the treatment of PPID.
Setting:	Unclear (all horses were maintained and medicated by their owners)
Study Population:	14 horses selected based on clinical signs of PPID and increased plasma ACTH (n=12) and/or ODST (n=4). Aged ≥20 years (n=11) and ≤19 years (n=3); 8 geldings and 6 mares.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=14 PPID cases treated with Vitex agnus castus extract for 2 – 6 months. Study design intended treatment duration to be 6 months, but due to deterioration in clinical signs, treatment was discontinued after 2 months (4 horses) or 4 months (4 horses), while 6 remained on treatment for the 6 month duration. Due to worsening of their clinical condition, 9 of the Vitex treated horses were subsequently treated with pergolide (0.002 – 0.006 mg/kg once daily per os) for 2 – 9 months (7 horses received pergolide for 3 months and 2 horses for > 4 months).
Outcome Measures:	Clinical signs and endocrine testing
Main Findings:	Only 1 of 14 horses remained stable and did not show clinical deterioration while treated with Vitex agnus castus extract. 8/9 horses subsequently treated with pergolide showed improvement in clinical signs. ACTH decreased in pergolide-treated horses: after 1 month of pergolide, ACTH was unchanged in 2/6 horses and reduced by ≥50% in 4/6 horses. After 3 months of pergolide, ACTH was within normal reference interval for 1/5, >35 but <60 pg/ml for 3/5 and remained elevated for 1/5 (between 240–363 pg/ml, but >50% reduction from pre-treatment values). Of the 4 PPID cases diagnosed based on abnormal ODST, 3 showed normal suppression after commencing pergolide treatment. One horse had pergolide treatment discontinued due to lethargy observed when administered 0.004 mg/kg daily for a 2 month period.
Interpretation of Results:	No description of case selection precluding assessment of selection bias. Very small sample size treated with pergolide. Did not use seasonally adjusted reference intervals for ACTH for diagnosis or monitoring response to treatment. Results demonstrate lack of efficacy of Vitex agnus castus in the treatment of PPID, and that pergolide is effective in improving endocrine parameters and clinical signs of PPID in the majority of treated horses.

Author, year: **Type of publication:**	Beech et al., 2009 Journal publication
Study Design:	Non-randomised controlled trial
Aim/Objective of the Study:	To measure plasma ACTH, α-MSH and insulin concentrations during various photoperiods between February and October in horses and ponies with and without PPID.
Setting:	Not stated – 36 privately owner pleasure horses/ponies; 6 PPID horses from research herd at University
Study Population:	No information provided regarding study population selection. PPID cases: n=15 with at least 2 "classical clinical signs" of PPID; 7 ponies (mean age 21.3 ± 4.3 years), 8 horses (mean age 24.5 ± 5.2 years); 5 geldings; 10 females. Controls: n=27 with no clinical signs of PPID; 13 ponies (mean age 7.1 ± 2.5 years), 14 horses (mean age 6.6 ± 1.6 years); 12 geldings, 14 females, 1 entire male.
Intervention Investigated:	*Control Group:* n=7 untreated PPID cases *Treatment Group:* n=8 (4/7 PPID ponies and 4/8 PPID horses) were being treated with oral pergolide. Pergolide dose ranged from 0.5mg daily in ponies to 3mg daily in horses.
Outcome Measures:	ACTH, α-MSH and insulin concentrations in different photoperiods in normal and PPID horses and ponies. Jugular blood samples collected during 8 photoperiods: 1) February 13th – March 2nd; 2) April 4th – 6th; 3) June 19th – 22nd; 4) August 6th – 7th; 5) August 14th – 17th; 6) September 4th – 6th; 7) September 26th – 28th and 8) October 16th – 18th. All plasma samples were assayed for ACTH and α-MSH. Insulin concentration was measured on plasma samples from 8 PPID group horses, 4 PPID group ponies, 6 control group horses and 6 control group ponies. Laboratory reference intervals were 9 – 35 pg/mL for ACTH and 10 – 40 μIU/mL for insulin. Reference concentration for α-MSH <90 pmol/L.
Main Findings:	Control group: Plasma ACTH significantly higher August – October than February – June. Plasma α-MSH concentrations significantly higher with increasing daylight. There were no significant seasonal changes in plasma insulin concentration except that log insulin concentration was lower in photoperiod 3 than in photoperiod 1. PPID group: Plasma ACTH and α-MSH concentrations were significantly higher in autumn photoperiods. Horses and ponies receiving pergolide, compared with those not receiving pergolide, had significantly less of an increase in plasma log α-MSH concentration in photoperiods 7 and 8, and significantly lower plasma log ACTH concentration during photoperiod 8.

Interpretation of Results:	Response to treatment with pergolide was not a study objective. Information on sample selection limited therefore unable to assess selection bias. Small sample size. Seasonally adjusted reference intervals indicated when using basal ACTH or α-MSH as diagnostic test for PPID. PPID cases treated with pergolide had lower ACTH compared to untreated cases during autumn.

Author, year: **Type of publication:**	Christen et al., 2018 Journal publication
Study Design:	Randomised placebo-controlled trial
Aim/Objective of the Study:	To evaluate faecal worm egg counts (FWEC) in horses with pre-clinical PPID before and after treatment with pergolide.
Setting:	Sanctuary for retired horses
Study Population:	n=48 horses; mean age 24.8 years (standard deviation 3.6 years; range 14–32 years); 22 geldings and 26 mares; divided in to pre-clinical PPID or healthy control groups based on basal ACTH: Pre-clinical PPID cases (n=24) with no obvious clinical signs of PPID but ACTH concentrations >35 pg/mL in July. Horses in pre-clinical PPID group randomly allocated to pergolide or placebo treatment group using simple randomisation from freely available online software. Controls (n=24) – healthy horses with ACTH concentrations below upper limit of reference interval.
Intervention Investigated:	Control Group: n=9 placebo-treated subclinical PPID cases (received same tablet as pergolide (Prascend, Boehringer Ingelheim) without the active ingredient for 3 months)
	Treatment Group: n=10 treated with pergolide (0.002 mg/kg once daily per os) for 3 months (1st October 2014 to 31st January 2015)
Outcome Measures:	Primary outcome measure was faecal worm egg count, measured at baseline, one month prior to treatment and after 3 month treatment period. ACTH measured before treatment (July) and after 3 months of pergolide or placebo treatment (January; n=19 horses as 5 animals in PPID group died of unrelated causes before second blood sample collected).
Main Findings:	Difference in FWEC after treatment in pergolide group compared with placebo-treated animals did not reach statistical significance. FWEC significantly lower after treatment in pergolide-treated animals compared to baseline (p=0.03) but not significantly different from baseline in placebo group. ACTH significantly lower following treatment with pergolide (p<0.01): median ACTH 75.2 pg/mL (range 39.1–195) at baseline and 25.9 pg/mL (7.4–50.1) following 3 months of pergolide treatment. No significant difference in baseline and post-treatment ACTH in placebo group: median 80.7 pg/mL (range 39.3–127) at baseline and 73.2 (37.8–479) following 3 months of placebo treatment.

Interpretation of Results:	Small sample size made smaller by high loss to follow-up for a short study. Methods do not state whether study was blinded. Treatment with pergolide resulted in significant reduction in ACTH levels that was not observed in the placebo-treated controls.

Author, year: **Type of publication:**	Donaldson et al., 2002 Journal publication
Study Design:	Case series
Aim/Objective of the Study:	To evaluate the response of horses with PPID to treatment with pergolide or cyproheptadine by evaluating clinical signs and plasma ACTH, insulin, and glucose concentrations before and after treatment.
Setting:	University referral hospital ambulatory service
Study Population:	n=27 PPID cases Records of horses evaluated for PPID at University of Pennsylvania Ambulatory service between June 1996 and November 2001. Criteria included plasma ACTH >50 pg/mL, at least 1 clinical sign, and evaluation before and after treatment. Horses were evaluated for 1-10 months (median 2 months).
Intervention Investigated:	*Control Group:* n=7 cases treated with oral cyproheptadine: 0.25 mg/kg as a total dose every 24 hours.
	Treatment Group: n=20 cases treated with oral pergolide: median oral dose was 3 µg/kg (0.003 mg/kg) once daily with a range of 1.7 to 5.5 µg/kg.
Outcome Measures:	Clinical signs and ACTH
Main Findings:	With pergolide treatment, there was a significant decrease in plasma ACTH, but not insulin or glucose concentration; 60% of horses reached a plasma ACTH within the normal reference interval. There was no significant change in any of the 3 parameters with cyproheptadine treatment. There was a direct correlation between pergolide dose and ACTH concentration after treatment, as well as between duration of treatment and ACTH concentration. Improvement in clinical signs was reported by 85% of owners of horses treated with pergolide and 28% of owners of horses treated with cyproheptadine. There was a significant decrease in the prevalence of laminitis following treatment with pergolide ($p<0.001$).
Interpretation of Results:	No details provided regarding selection of study population, nor treatment allocation, which was not randomised. Selection criteria for treatment not reported. Long study period yet short duration of follow-up. Subject to inherent biases affecting studies that rely on retrospective review of clinical records. Pergolide was clearly shown to be more effective than cyproheptadine in improving both clinical signs and ACTH, with 60% of pergolide-treated horses having normal ACTH concentration after treatment and 85% reported to have an improvement in clinical signs.

Author, year: **Type of publication:**	Dunkel et al., 2014 Journal publication
Study Design:	Case series
Aim/Objective of the Study:	To describe historical and clinicopathological findings, progression and outcome in horses and ponies with severe hypertriglyceridaemia secondary to an endocrine disorder.
Setting:	Cases presented to or treated by various University referral hospitals in the UK and USA.
Study Population:	n=6 PPID cases Age range 17 – 28 years; 2 mares, 3 geldings and one case where sex was not reported. PPID diagnosed on clinical signs and ACTH concentrations (n=4) or overnight dexamethasone suppression test (n=2).
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=5 cases treated with oral pergolide; doses ranged from 0.7 – 8 µg/kg daily per os (median starting dose 3.8 µg/kg once daily per os)
Outcome Measures:	Clinical signs and ACTH
Main Findings:	Clinical improvement was observed in the majority of treated horses (4/5), short term improvement in ACTH was reported in 2/3 horses. Adverse effects (depression, anorexia, and heart murmur) observed in 1 horse treated with compounded pergolide (3.8 µg/kg per os once daily), not observed on subsequently reduced dose of pergolide.
Interpretation of Results:	Small descriptive case series treated at/by teaching/referral hospitals, and all cases also had concurrent hypertriglyceridaemia so results are not likely to be generalisable all PPID cases. Variable follow-up duration and in terms of clinical examination findings and laboratory analyses reported. Subject to inherent biases affecting studies that rely on retrospective review of clinical records. Severe hypertriglyceridaemia can develop secondary to PPID, and successful treatment of PPID with pergolide may resolve this without primary treatment of hypertriglyceridaemia.

Author, year: **Type of publication:**	Froin et al., 1998 Conference abstract
Study Design:	Case series
	To study the influence of pergolide treatment on plasma ACTH and cortisol values of horses with an adenoma of the pars intermedia and control horses.
Setting:	Private referral practice
Study Population:	ACTH and cortisol measured in 42 PPID cases and 55 healthy controls. n=5 PPID cases treated with pergolide and followed for up to 12 months

Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=5 treated with pergolide (1.7 µg/kg once daily per os)
Outcome Measures:	Treatment response based on clinical signs, ACTH and cortisol
Main Findings:	All treated horses showed clinical improvements, after a minimum period of 3 months of pergolide treatment. All horses returned to their pre-illness activity level after 3-12 months of treatment. ODST results returned to normal in 3, basal cortisol reduced in 1 and ACTH reduced in another.
Interpretation of Results:	Small study, incompletely reported as an abstract, with variable laboratory analyses reported for assessing response to treatment. No information on case selection reported therefore unable to assess selection bias. A further 37 PPID cases identified via elevated basal ACTH but no details provided regarding why these cases were not treated/followed-up. Overall positive response to pergolide therapy at quite a low dose in 5 PPID cases.

Author, year: **Type of publication:**	Gehlen et al., 2014 Journal publication
Study Design:	Case control study
Aim/Objective of the Study:	To examine insulin and glucose metabolism in horses with PPID, to investigate the effect of pergolide treatment on insulin and glucose metabolism and to determine whether pergolide has an effect on laminitis by lowering hyperinsulinaemia.
Setting:	Not reported
Study Population:	n=38 PPID cases, diagnosed based on basal ACTH ≥50 pg/mL measured in January plus advanced clinical signs of PPID. Study population included 17 ponies, 16 Warmbloods, 4 Thoroughbreds, and 1 draught horse; 22 mares and 16 geldings; mean age 24 years (range 16 – 38 years).
Intervention Investigated:	*Control Group:* n=25 untreated PPID cases
	Treatment Group: n=13 cases treated with oral pergolide for at least 3 months; doses ranged from 0.5-2 mg/day per os
Outcome Measures:	Insulin, insulin resistance (IR) and ACTH were measured along with triglyceride, fructosamine and glucose levels, and number of animals with historical laminitis episodes.
Main Findings:	Difference in ACTH levels between pergolide-treated and non-treated horses not shown but reported as not statistically significant, however levels tended to be lower in treated animals. Overall, 39.5% (n=15/38) showed alterations of glucose and insulin metabolism. There were no significant differences between groups for insulin, glucose, triglyceride, fructosamine or IR and these were not different between treated and untreated animals. No correlation between glucose metabolism and ACTH levels or ACTH levels and age. Laminitis was observed in both groups.

Interpretation of Results:	Stated study aim of determining whether pergolide affects insulin and therefore laminitis incidence is not addressed. Similarly, without having assessed insulin and glucose parameters before and after pergolide treatment, the study aim of investigate the effect of pergolide treatment on insulin and glucose metabolism has not been achieved. No description of the sampling frame or case selection, precluding assessment of selection bias. Selection criteria for pergolide treatment not reported. Possible bias towards more severe cases receiving pergolide which may have influenced findings. Variable doses without bodyweight information also make comparison or extrapolation of findings difficult. Provides very limited information regarding the effect of pergolide treatment on insulin and glucose metabolism and ACTH levels.

Author, year: **Type of publication:**	Horn et al., 2019 Journal publication
Study Design:	Retrospective cohort study
Aim/Objective of the Study:	To describe clinical features of PPID at lower southern latitudes in Australia and to investigate factors associated with survival, laminitis and insulin dysregulation (ID) in equids diagnosed with PPID.
Setting:	Eight veterinary institutions in five regions of Australia; 57% of PPID cases were first opinion and 43% were referral hospital cases, diagnosed between 2002 – 2018
Study Population:	n=274 PPID cases, diagnosed between 2002 and 2018, based on basal ACTH (n=263), ODST (n=9), TRH stimulation test (n=12) or post mortem examination (n=2). Reported data indicate a small number of cases were diagnosed using >1 of the tests above, but no further details provided. ACTH cut-off values adjusted by season and location: northern Queensland: autumn 101 pg/mL; non-autumn 67 pg/mL southern Western Australia: autumn 94 pg/mL; non-autumn 43 pg/mL Tasmania: autumn 75 pg/mL; non-autumn 46 pg/mL southern South Australia, Victoria and southern Queensland: autumn 77.4 pg/mL; non-autumn 29.7 pg/mL Season of diagnosis was spring for 22.3%, summer for 19.0%, autumn for 38.5% and winter for 20.1% of cases. Study population included 48.1% ponies, 12.6% Thoroughbreds, 10.7% Warmbloods, 9.5% Arabs, and 50 other horse breeds (19.1%) (based on 262 cases with breed recorded); 42.0% mares, 53.3% geldings and 4.7% stallions (based on 255 cases with sex recorded); median age at diagnosis 21 years (range 8 – 42 years).

	71.9% of cases had follow-up information, with duration of follow-up ranging from 0 – 85 months (median 11 months). Proportion of pergolide-treated cases with follow-up data not reported.
Intervention Investigated:	*Control Group:* n=29 PPID cases, presumed to be untreated but not reported in publication and not clear how many of these cases had follow-up
	Treatment Group: n=218 cases treated with oral pergolide; doses ranged from 0.5 – 12.5 µg/kg daily per os (median starting dose 2 µg/kg once daily per os). Where reported (n=119/218 cases), 45.6% were treated with a liquid formulation of pergolide and 54.6% were treated with a tablet form. Pergolide dose was increased for 61 cases, with final pergolide dose ranging from 0.5 – 16 µg/kg daily per os (median final dose 2 µg/kg daily per os). The proportion of cases treated with a tablet form of pergolide increased by the final re-examination (78.1%; n=57/73) compared to 21.9% of cases treated with liquid pergolide.
Outcome Measures:	Laminitis (diagnosed by radiography or histology), insulin dysregulation clinical signs, ACTH concentrations and survival.
Main Findings:	92 pergolide-treated cases (64.3%; denominator not reported but calculated as 143 and presumed to represent total number of pergolide-treated horses with follow-up data) had veterinary-reported clinical improvement at re-examination. Only 1 untreated case (6.7%) was reported to have clinical improvement observed at re-examination. ACTH concentrations were first retested for 62.9% of cases at a median of 4 months (range 1 – 6 months), with up to 3 further re-tests reported for a small proportion of cases, performed at decreasing frequency (median 12.5 months between 1st and 2nd tests up to median of 25 months between the 3rd and 4th tests). Of 117 pergolide-treated cases, 44.4% had ACTH concentrations within reference intervals at repeat testing, compared to 0% of untreated cases. However, data presented indicates that not all pergolide-treated cases had normal ACTH concentrations at the first re-examination, nor across all repeat tests. Pergolide treatment was associated with increased odds of survival (odds ratio 3.8; p=0.04).
Interpretation of Results:	Provides some comparison between pergolide-treated and untreated cases. Subject to inherent biases affecting studies that rely on retrospective review of clinical records. 28% loss to follow-up which may introduce bias if not occurring at random. Several variables evaluated had moderate-high amount of missing data, with only 71 cases included in the final multivariable logistic regression model for prediction of survival. Seasonally adjusted ACTH cut-off values utilised for PPID case definition were not available for the first 10 years of the study period, and may therefore differ from diagnostic cut-off values utilised by treating veterinary surgeons at the time of case presentation. Selection criteria for pergolide treatment not reported. Possible bias towards more severe cases receiving pergolide which may have influenced findings. Conversely, more severe cases may have been more likely

	to be euthanased at the time of, or within short time period following, diagnosis. Variable follow-up duration and intervals at which laboratory analyses performed. Duration of pergolide treatment prior to veterinary re-examination not reported, therefore unable to determine time period over which clinical improvement was observed. Pergolide treatment resulted in clinical improvement in in 64% horses, normalisation of ACTH concentration in ~44% following treatment and was positively associated with survival.

Author, year: **Type of publication:**	Innerå et al., 2013 Journal publication
Study Design:	Uncontrolled, non-randomised field trial
Aim/Objective of the Study:	To compare hair follicle stages in PPID-affected horses with hypertrichosis with normal aged horses (controls) and to compare hair follicle stages in PPID-affected horses after 6 months of treatment with pergolide mesylate with those of control horses.
Setting:	University referral hospital and 5 client-owned PPID cases kept at home premises
Study Population:	n=8 PPID-affected horses (5 mares and 3 geldings; mean age 24 years; range 20-29 years) and n=4 normal, age-matched control horses (3 mares and 1 gelding; mean age 25 years; range 21-29 years). PPID cases diagnosed based on hypertrichosis and ODST, and were included in study population for pergolide field trial (Anon 2011). Control horses had no history of abnormal hair coat or delayed shedding and had normal ODST results.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=8 treated with oral pergolide (2 µg/kg once daily per os), and increased to 4 µg/kg once daily for all 8 cases as ODST test results remained abnormal after 3 months of treatment.
Outcome Measures:	Skin biopsy results – the number of hair follicles in anagen (A) or telogen (T) was counted using transverse sections, and mean percentages of A and T stages of hair follicles in PPID cases post-treatment with pergolide were calculated. Clinical scoring of hypertrichosis (0 = normal, no unusual hair growth; 1 = regional hair coat changes, long hair growth restricted to discrete areas; 2 = generalised hair coat changes (slightly to moderately long hair coat that fails to shed out as in previous years); 3 = severe hair coat changes (severely long and/or curly hair coat over the entire body that fails to shed)).
Main Findings:	Pre-treatment biopsies had a greater percentage of A follicles (neck 96%, rump 95%) and a lower percentage of T follicles (neck 4%, rump 5%) in PPID-affected horses than in control horses (A, neck 15%, rump 25%; and T, neck 85%, rump 75%). After 3 months of pergolide treatment, PPID cases showed improved attitude, decreased sweating, and decreased polyuria and polydipsia, and a decreased in median hair coat score from 3 pre-treatment to 2.5.

	After 6 months treatment with pergolide, 50% of PPID cases had normal ODST results, all PPID-affected horses had improved shedding, median hair coat score had decreased to 1, and the percentages of A follicles (neck 69%, rump 70%) and T follicles (neck 31%, rump 30%) were not different from untreated control horses (A, neck 68%, rump 82%; and T, neck 32%, rump 18%).
Interpretation of Results:	Information on sample selection limited therefore unable to assess selection bias. Small sample size. Study gives an objective indicator of treatment success un-reliant on owner opinion. Findings indicate excessively long hair coat in horses with advanced PPID is a consequence of persistence of hair follicles in the anagen stage. Treatment with pergolide improved hair coat shedding and reduced the percentage of anagen follicles in PPID-affected horses, and normalisation of ODST was achieved in 50% of pergolide-treated PPID cases.

Author, year: **Type of publication:**	Love 1993 Journal publication
Study Design:	Case series
Aim/Objective of the Study:	To describe clinical presentation and outcome following treatment in 5 PPID cases.
Setting:	University referral hospital
Study Population:	n=5 PPID cases; age range 13 – 34 years; all British native breed ponies referred to Glasgow University during a 2 year period starting in January 1990. PPID diagnosed based on TRH stimulation test measuring cortisol.
Intervention Investigated:	*Control Group:* n=1 untreated; n=4 treated with oral cyproheptadine (incremental increased dose from 0.6 mg/kg$^{0.75}$ to 1.2 mg/kg$^{0.75}$)
	Treatment Group: n=1 treated with oral pergolide; following poor results with cyproheptadine treatment and 2 ponies being euthanased, 1 of the remaining 2 treated ponies was subsequently treated with oral pergolide (3mg daily for 6 weeks, 1mg daily for 6 weeks, then 1mg every other day thereafter).
Outcome Measures:	Clinical signs
Main Findings:	Five ponies were diagnosed with PPID. Four of the 5 ponies were treated with cyproheptadine with a satisfactory response in only one pony. One pony was treated with pergolide after unsuccessful treatment with cyproheptadine and showed marked clinical improvement after 3 weeks, which was sustained during 6 months of follow-up. Temporary anorexia (10 day duration) was noted after initiation of pergolide treatment in this animal, but improved within 3 weeks.
Interpretation of Results:	No information on case selection reported therefore unable to assess selection bias. Small sample size from referral hospital population. Single pony treated successfully with pergolide after treatment failure with cyproheptadine. Temporary anorexia in one pergolide-treated pony when on high dose (3mg daily).

Author, year: **Type of publication:**	McFarlane et al., 2017 Journal publication
Study Design:	Uncontrolled, non-randomised clinical trial
Aim/Objective of the Study:	To evaluate the pharmacokinetic and pharmacodynamic properties of pergolide in horses with PPID after long-term oral administration.
Setting:	University referral hospital research herd
Study Population:	n=6 PPID cases (mean age 24.3 years; range 18 – 28 years; 3 geldings and 3 mares; 3 Quarter Horses, 2 Tennessee Walking Horses and 1 Standardbred cross). PPID cases diagnosed based on clinical signs and TRH stimulation test.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=6 treated with oral pergolide once daily: 1 mg/day per horse (~0.002 mg/kg/day) for 2 months and then 2 mg/day per horse (~ 0.004 mg/kg/day) for an additional 4 months. Pergolide tablet was dissolved in 3-5 mL of water then mixed in feed.
Outcome Measures:	Steady-state pharmacokinetic properties of pergolide in PPID cases and duration of ACTH regulation and duration of quantifiable drug in the plasma of horses after discontinuation of pergolide following long-term administration.
Main Findings:	After discontinuing treatment, pergolide could be quantified in plasma for 2-7 days and ACTH levels significantly increased in all horses after 14 days following cessation of treatment. Basal (but not TRH stimulated) ACTH concentration was significantly lower at weeks 16, 20, and 24 after initiation of pergolide treatment compared to pre-treatment values. After 2 months of the initial dose, ACTH levels in all horses were above the reference interval and ACTH concentration remained above the seasonal reference interval (>100 pg/mL) in all horses after a subsequent increase in pergolide dose to ~ 0.004 mg/kg/day.
Interpretation of Results:	Information on sample selection limited therefore unable to assess selection bias. Small sample size with no comparison group. Pergolide effectively decreased ACTH concentration in all horses, though not to within seasonally adjusted reference intervals. After discontinuing pergolide treatment, ACTH concentration increased by 50% in 3/6 horses by 2 days and 6/6 horses by 10 days. Pergolide was detectable in all horses at 2 days and in none at 10 days after the last dose. Results support once daily dosing with pergolide is appropriate for the treatment of PPID cases.

Author, year: **Type of publication:**	Orth et al., 1982 Journal publication
Study Design:	Case series

Aim/Objective of the Study:	To study plasma concentrations of pro-OLMC peptides in 10 normal horses under basal conditions and in 2 PPID cases under basal conditions, during various tests of pituitary-adrenal function, and after the administration of dopamine and dopaminergic agonists.
Setting:	Not stated
Study Population:	n=10 normal control horses n= 2 PPID cases Case 1: 7-year-old mare with clinical signs consistent with advanced PPID. Blood tests were performed, and the horse was then euthanased because no effective treatment was available. Post mortem revealed a pars intermedia adenoma and hyperplastic adrenal glands. Case 2: 12-year-old mixed breed pony mare with clinical signs of PPID, including laminitis. Blood tests were performed, and the pony was successfully treated (therapy not specified). Laboratory tests performed in PPID cases included cortisol, ACTH, and other pro-OLMC peptides including αMSH, βMSH, corticotrophin-like intermediate lobe peptide [CLIP], β-lipotropin [β-LPH], and β-endorphin), low and high dose dexamethasone suppression tests, ACTH stimulation, glucose tolerance, and insulin tolerance. Additional tests performed in Case 2 included the response of plasma cortisol and pituitary peptides to insulin, vasopressin, dopamine-HCL, bromocriptine, or pergolide mesylate. Normal horses: a single blood sample was taken between 8 and 10 am to establish a standard for pituitary peptide concentrations.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=1 PPID case • Intravenous dopamine hydrochloride, 4 µg/kg/min for 150 minutes • Oral and subcutaneous bromocriptine, 100 mg total dose (dissolved in 2ml 40% ethanol) • Oral pergolide mesylate, 5 mg total dose alone and in combination with dexamethasone (capsule form of pergolide provided by Eli Lilly)
Outcome Measures:	Pro-OLMC peptides basally and in response to standard tests of pituitary-adrenal function.
Main Findings:	Both PPID cases had elevated basal and pro-OLMC peptides were disproportionately increased. Both PPID cases were glucose intolerant. Agents that affect secretion from the pars distalis (glucocorticoids, vasopressin and hypoglycaemia) had little or no effect on pro-OLMC secretion. Dopamine and dopamine agonists (bromocriptine, pergolide) inhibited pro-OLMC secretion from the pars intermedia in a single PPID case. Dopamine caused a rapid fall in hormone levels. Bromocriptine and pergolide caused rapid and sustained decreases in hormones to normal levels.
Interpretation of Results:	Only included 2 PPID cases with very advanced disease and concurrent insulin resistance. Pharmacological intervention study on a single case of PPID provided details of the endocrinological effect and duration of action of pergolide. Pergolide mesylate (5 mg, 0.014 mg/kg) was compared to dopamine and bromocriptine

	and was found superior due to prolonged duration of action and ease of administration. There were clear and marked reductions in ACTH, α-MSH, β-endorphin and CLIP for 48 hours following a single dose of pergolide.

Author, year: **Type of publication:**	Pease et al., 2011 Journal publication
Study Design:	Case control study
Aim/Objective of the Study:	To determine whether pituitary glands of PPID-affected horses are larger than those of aged horses without signs of PPID, whether the size difference can be detected using computed tomography (CT) imaging and whether pituitary gland size decreased in subset PPID cases after treatment with pergolide.
Setting:	University referral hospital research herd
Study Population:	n=8 PPID cases (mean age 24 ± 4 years; range 18-28 years). PPID diagnosed based on hypertrichosis and ODST. n=3 aged control (PPID negative) horses (mean 24 ± 5 years; range 20-29 years). Computed tomography (CT) imaging twice 6 months apart in 10 horses, 6 treated with pergolide.
Intervention Investigated:	*Control Group:* n=2 untreated PPID cases *Treatment Group:* n=6 PPID cases treated with oral pergolide: 1 mg/day per os for 6 months (~ 2.3 µg/kg/day based on mean weight of 435kg); increased to 2 mg/day for 3 horses after 3 months due to ODST results remaining abnormal.
Outcome Measures:	Pituitary gland height, width, length, weight, response to treatment with pergolide.
Main Findings:	On initial examination, pituitary glands of PPID horses were larger in height ($p<0.002$) and width ($p<0.01$) than controls, but the difference in length was not significant ($p=0.06$). After 6 months of pergolide treatment of PPID horses, pituitary gland length increased ($p<0.03$), but height and width were not different from pre-treatment values. All 6 pergolide-treated horses showed clinical improvement (less lethargy, improved hair coat shedding and weight gain) after 3 months, and ODST results were normal in 3/6 horses. After 6 months of pergolide treatment, improved shedding of hair coat was observed in all treated horses, although 1 horse with a history of chronic laminitis had an exacerbation of laminitis when spring pasture became available. ODST results were normal in 3 horses after 6 months, including 2 horses that had not been normal at 3 months. One of the non-treated PPID horses remained fairly healthy (with persistent hypertrichosis) during the 6-month study period, whereas the other horse had exacerbations of chronic laminitis and developed fungal keratitis prompting unilateral enucleation. ODST results in the 2 untreated PPID cases remained abnormal.
Interpretation of Results:	Sample selection likely to introduce bias, and small sample size. Selection criteria for pergolide treatment not reported. ODST follow-up at 6 months was in August-September and likely to be

	affected by season. Relevance of CT findings to clinical practice limited, but study showed CT may be a useful imaging modality to determine pituitary gland size of PPID-affected horses if facilities for contrast enhancement and general anaesthesia are available. Treatment with pergolide does not affect pituitary gland size, but resulted in improvement in ≥1 clinical sign in all cases and 5/6 cases had normal ODST results after either 3 or 6 months.

Author, year: **Type of publication:**	Perkins et al., 2002 Journal publication
Study Design:	Retrospective cohort study
Aim/Objective of the Study:	To determine the value of monitoring plasma ACTH levels during treatment of PPID with either cyproheptadine or pergolide.
Setting:	New York State Diagnostic Laboratory
Study Population:	Questionnaire survey sent to submitting veterinary surgeon where horses had ACTH >35 pg/mL. Duration of follow-up: median time was 2 months (1-15 month range) between baseline and first recheck, and between first recheck and second recheck.
Intervention Investigated:	*Control Group:* n=32 PPID cases treated with cyproheptadine (23 horses and 9 ponies): n=5 on 225-250 mg/day (either 0.5 mg/kg once daily or 0.25 mg/kg every 12 hours) and n=27 on 450-500 mg/day
	Treatment Group: n= 10 PPID cases treated with pergolide (7 horses and 3 ponies): n=7 on 0.75-1.0 mg/day (~0.0017 mg/kg/day once daily per os); n=1 on 1.5-2mg/day and n=2 on 0.38-0.5mg/day.
Outcome Measures:	ACTH and clinical improvement based on survey of veterinary surgeons
Main Findings:	Overall including both treatment group, ACTH decreased significantly from baseline to first recheck in all PPID cases, but not between first and second rechecks. 90% of pergolide and 81% of cyproheptadine treated horses had an improvement in ≥1 clinical sign. 90% of pergolide-treated horses presented with hypertrichosis, and of these 44% (n=4/9) showed an improvement after treatment. Decreases in ACTH were associated with improvements in hypertrichosis. Pergolide-treated horses showed normalisation to within reference interval for ACTH at first recheck in 10% of horses and 40% at second recheck. Improvement (but not normalisation) in ACTH was seen in 60% of horses at first and second rechecks in pergolide-treated horses.
Interpretation of Results:	Major limitation is that study is based on laboratory sample data and the authors did not examine the horses, but relied on veterinary-reported questionnaire data. Subject to inherent biases affecting retrospective studies and where data not accurately recorded in clinical records, there may be recall bias. No indication of vet response rate is provided. Results are presented as percentage of horses with decreases in ACTH and so limit the ability to interpret the statistics. Seasonality is not taken into consideration and will have affected the results.

	Pergolide treatment resulted in improvement in at least one clinical sign in 90% horses and a decrease in ACTH in 60% following treatment.

Author, year: **Type of publication:**	Peters et al., 1995 Journal publication
Study Design:	Case series
Setting:	Private practice
Aim/Objective of the Study:	To assess the efficacy of low-dose pergolide therapy in horses and ponies with adenomas of the pituitary gland.
Study Population:	n=9 PPID cases; 5 horses (age range 18 – 32 years) and 4 ponies (age range 16 – 30 years). Cases presented between May 1993 and April 1995 for evaluation of clinical signs indicative of PPID. PPID diagnosed based on ODST.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=9 PPID cases treated with oral pergolide (Permax® tablets) at 0.0017 mg/kg a day (1.7 µg/kg), divided to be administered twice daily. Average duration of treatment 13.6 months (range 1 – 23 months).
Outcome Measures:	Clinical signs and ODST
Main Findings:	Eight of nine horses (88.9%) showed clinical improvement within 14 to 35 days (average 22.75 days), and seven of those had ongoing improvement for 5 to 52 weeks (average 21 weeks; after which response to treatment appeared to stabilise). ODST showed improvement in 5 of 7 horses (71.5%) in 1.75-4.5 months (average 3.05 months); normalisation in ODST in 2 of 7 horses (28.6%). Hyperglycemia resolved in all 5 horses/ponies that presented with elevated glucose at baseline. Two of three horses presenting with laminitis were reported to be markedly improved within 2 weeks after pergolide treatment started. One horse showed no clinical improvement but did show improved ODST results. No adverse effects of treatment were observed.
Interpretation of Results:	No information regarding case selection therefore unable to assess selection bias. Small sample size with no comparison group. Monitoring was by owners and not by veterinary surgeons, for an average time of 13.6 months (range 1 – 23 months); therefore potential for introduced biases/errors from owner-reported data. Clinical signs improved in 8/9 (89%) and 5/7 horses showed improvement of the ODST. Several other clinicopathological or endocrinological variables improved in the majority of cases. Improvements occurred in a time frame of 2 – 5 weeks. The authors report favourable clinical results and lack of side effects at this lower dose rate of pergolide.

Author, year: **Type of publication:**	Pongratz et al., 2010 Journal publication

Study Design:	Retrospective cohort study
Aim/Objective of the Study:	To evaluate the long-term effects of pergolide therapy in PPID cases by surveying owners using a questionnaire.
Setting:	19 PPID cases from University referral hospitals and 19 from first opinion vets in Switzerland
Study Population:	n=38 PPID cases, diagnosed between 1999 – 2009. PPID diagnosed by ODST (n=13/38), elevated plasma ACTH (n=20/38) or both tests (n=4/38). One horse was diagnosed solely on the basis of clinical signs. Mean age 22 years (range 13-36 years); 22 Warmbloods, 11 Cobs and 5 ponies; 14 mares, 23 geldings and 1 stallion.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=38 treated with oral pergolide; 0.001 – 0.002 mg/kg per os once daily (n=23 on 0.001mg/kg and n=11 on 0.002 mg/kg; dose not reported for other 4 cases).
Outcome Measures:	Owner-reported questionnaire data on clinical signs
Main Findings:	Clinical improvement reported for 88.6% of cases (n=31/35). Improvement in clinical signs noted by owners after starting pergolide treatment within 1-2 weeks (18.4%; n=7/38); within 2-4 weeks (47.4%; n=18/38) or after 8 weeks (21.1%; n=8/38). Following pergolide treatment, prevalence was significantly lower (all $p<0.05$) for hypertrichosis (before 86.5%, n=33/38; after 23.7%, n=9/38), hyperhidrosis (before 63.2%, n=24/38; after 18.4%, n=7/38), poor performance (before 55.3%, n=21/38; after 79%, n=3/38), lethargy (before 57.9%, n=22/38; after 7.9%, n=3/38), polyuria/polydipsia (before 39.5%, n=15/38; after 13.2%, n=5/38), muscular atrophy/lordosis (before 50.0%, n=19/38; after 28.9%, n=11/38), weight loss (before 34.2%, n=13/38; after 10.5%, n=4/38), fat redistribution (before 36.8%, n=14/38; after 7.9%, n=3/38), ataxia (before 28.9%, n=11/38; after 7.9%, n=3/38), skin infections (before 26.3%, n=10/38; after 10.5%, n=4/38) and laminitis (before 39.5%, n=15/38; after 7.9%, n=3/38; 12/15 cases with previous history of laminitis had no further reported episodes following commencement of pergolide treatment). Average duration of treatment was 13.8 months (range 1 – 38 months). 90% of owners (n=34/38) were extremely satisfied with pergolide treatment. Adverse effects were reported in 28.9% of cases (n=11/38); most frequently anorexia (15.8%, n=6/38), lethargy (10.5%, n=4/38), and diarrhoea (5.3%, n=2/38), and 73.3% of affected horses (n=8/11) showed adverse effects within the first 4 weeks of treatment. All adverse effects were self-limiting with a constant daily dosage, with the exception of 1 case where pergolide dose was reduced from 1.5 µg/kg/day to 1 µg/kg/day.
Interpretation of Results:	Relied on owner-reported health data. May be subject to selection, response and recall biases (variable and long time period between diagnosis and data collection, e.g. 5/38 cases diagnosed >6 years

	previous to completion of questionnaire). Moderate owner response rate – no comparison with non-responders, and very low veterinary response rate (8%), though unclear which data were veterinary-reported. The vast majority of owners were satisfied with pergolide treatment, and clinical improvement was observed in 89% of cases.

Author, year: **Type of publication:**	Rendle et al., 2013 Conference abstract
Study Design:	Retrospective cohort study
Aim/Objective of the Study:	To evaluate ACTH response in horses treated with pergolide and to investigate factors that may influence response to treatment.
Setting:	Records of submissions to a single private practice diagnostic laboratory (The Liphook Equine Hospital Laboratory)
Study Population:	n=2,122 PPID cases Laboratory records reviewed for cases in which ACTH concentration was measured before and after instigation of pergolide treatment between January 2007 and December 2012.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: all PPID cases where pergolide was commenced after PPID diagnosis; no details of dose presented.
Outcome Measures:	Reduction of ACTH concentration of ≥75%, or a return of ACTH concentration to within seasonally adjusted reference intervals.
Main Findings:	At the first follow-up, improvement in ACTH was identified in 54.8% of cases and return of ACTH concentration to within seasonal adjusted reference intervals was identified in 28% of cases. Equids with a higher ACTH concentration pre-treatment with pergolide were more likely to improve (odds ratio [OR] 1.01, 95% CI 1.00-1.02; p=0.03), but less likely to return to within the reference interval (OR 0.97; 95% CI 0.96–0.98; p<0.001). Older equids were less likely to improve (OR 0.56, 95% CI 0.46-0.67; p<0.001). Improvement was more likely after a cumulative pergolide dose of 50 mg (OR 1.59, 95% CI 1.10-2.29; p=0.01) and duration of treatment was positively associated with treatment response (p=0.04). A daily dose of >0.5 µg/kg was less likely to be associated with a reduction in ACTH concentration (OR 0.86, 95% CI 0.77-0.96; p=0.009).
Interpretation of Results:	Major limitation is that study is based on laboratory sample data and the authors did not examine the horses, but relied on clinical data recorded on laboratory submission forms. Subject to inherent biases affecting retrospective studies. Unclear as to how cumulative and estimated daily pergolide dose were calculated. Results show pergolide treatment is effective in reducing ACTH in the majority of PPID cases, although degree of improvement may vary depending on signalment and dose-related factors.

Author, year: **Type of publication:**	Rendle et al., 2018 Conference abstract

Study Design:	Retrospective cohort study
Aim/Objective of the Study:	To assess the efficacy of a palatable pergolide paste in PPID cases considered to be refractory to treatment with a licensed pergolide tablet formulation.
Setting:	Not reported
Study Population:	n=19 PPID cases PPID cases selected for inclusion based on history of being refractory to treat with a licensed pergolide tablet formulation. Retrospective review of clinical records of 19 ponies with clinical signs of PPID and ACTH concentration >50 pg/mL in July (10 ponies) or >100 pg/mL from August to October (9 ponies). All ponies were re-examined after 1 month of treatment, in October, November and after 6 months of treatment.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=19 administered palatable pergolide paste; dose not reported
Outcome Measures:	Clinical signs, ACTH and insulin concentrations
Main Findings:	Median ACTH decreased significantly following treatment with the pergolide paste (from 206 pg/mL to 25 pg/mL in autumn cases, and from 110 pg/mL to 37 pg/mL in non-autumn cases). ACTH concentrations returned to within seasonally adjusted reference intervals for 74% of cases (n=14/19). No significant difference in insulin concentration observed following treatment. Not possible to interpret improvement in clinical signs following treatment: abstract states "*clinical signs improved markedly, mildly, did not improve or deteriorated in 31%, 12%, 43%, 4% and 10%, respectively*" – the number of categories does not correspond to the number of proportions reported, therefore unable to determine which proportion relates to which category. Inappetence was reported in 2 ponies (11%), which resolved after reduction of pergolide dose.
Interpretation of Results:	Limited information provided in conference abstract. Limited information regarding case selection, therefore unable to assess selection bias: ponies appear to have been recruited from private practice(s) although this is not stated. Small sample size. Subject to inherent biases affecting studies that rely on retrospective review of clinical records. Reasonable duration of follow-up, however information on dosing not reported. ACTH concentration improved significantly to within reference ranges in the majority of ponies. It is not possible to determine improvement in clinical signs from the data reported.

Author, year: **Type of publication:**	Rohrbach et al., 2012 Journal publication
Study Design:	Retrospective cohort study
Aim/Objective of the Study:	To identify trends in the diagnosis of PPID in horses presented to veterinary teaching hospitals and to identify clinical and laboratory factors associated with long-term prognosis, client perception of treatment, and quality of life in a subset of PPID cases.

Setting:	Veterinary teaching hospitals
Study Population:	Recorded diagnoses of PPID or synonym retrieved from database for 15 veterinary teaching hospitals from 1st January 1993 to 30th June 2004: 217 PPID cases identified, plus further 14 cases from clinical records at another University veterinary referral hospital. n=44 PPID cases meeting inclusion criteria, of which 34 cases had follow-up. Mean age of PPID cases at diagnosis 21.6 ± 6.6 years (n=36), 28 geldings and 16 mares; 35 horses and 9 ponies. Most frequent method of PPID diagnosis was by presence of hypertrichosis only in 15 (34%), and by hypertrichosis and ODST test results in 10 (23%) of cases.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: Treatments administered after diagnosis were available for 34/44 (77%) of study horses. Pergolide was given to 16/34 (47%), cyproheptadine to 12/34 (35%), and both pergolide and cyproheptadine to 6/34 (18%).
Outcome Measures:	Owner based telephone follow-up over 6 month period from July to December 2004. For owner-reported clinical response, a positive response to treatment was defined as improvement in ≥ 1clinical sign at 2 months post-treatment without an increase in severity for any other signs exhibited at presentation.
Main Findings:	Across all participating veterinary teaching hospitals, proportional diagnoses of PPID increased from 0.25/1,000 hospital records in 1993 to 3.7/1,000 in 2002. For the majority of horses, clinical signs did not change over the first 2 months post-treatment. Owners providing information on type of treatment and response to treatment at 2 months (n=22) reported positive response rates of 40% for pergolide (n=4/10) and 29% for cyproheptadine (n=2/7), whereas 3/5 (60%) of horses treated with both drugs had a positive response. Clinical signs and clinicopathological data were not associated with survival, and 50% of cases were alive 4.6 years after diagnosis.
Interpretation of Results:	Significant loss to follow-up, even though study used a small convenience sample – likely to have introduced bias. Majority of results presented relied on owner-reported health data. Case selection for follow-up poorly described. May be subject to selection, response and recall biases (variable and long time period (6 months – 12 years) between diagnosis and data collection). PPID cases presented to veterinary teaching hospitals may not represent cases in the field. Small sample size of animals treated solely with pergolide. Pergolide had a better owner-reported outcome after 2 months of treatment than cyproheptadine, and the proportion of treated cases with clinical improvement was even greater when pergolide used in combination with cyproheptadine, but very small numbers and no direct comparison between treatment groups presented (though discussion section mentions difference between groups not statistically significant).

Author, year:	Schott et al., 2001 Conference abstract

Type of publication:	
Study Design:	Uncontrolled, non-randomised field trial
Aim/Objective of the Study:	To compare clinical and/or endocrine responses in horses with PPID that are treated with cyproheptadine, pergolide, or not treated.
Setting:	Collaboration between a University referral hospital and local veterinarians
Study Population:	Horses with naturally occurring PPID presented to Michigan veterinary surgeons and enrolled in this field study administered by Michigan State University from 1997 to 1999. Enrollment criteria included clinical signs and diagnostic low-dose ODST or TRH stimulation test (measuring cortisol). n=77 PPID cases enrolled; mean age 22.8 years (range 12–34 years); most numerous breeds were Crossbreds (n=16), ponies (n=15), Morgan (n=14), Quarter Horse (n=8), Arab (n=7), and Thoroughbred (n=4); 37 (48%) mares, 38 (49%) geldings, and 2 (3%) stallions. Follow-up evaluations undertaken at 6 to 12 months, with only 32 cases having follow-up post-treatment.
Intervention Investigated:	*Control Group:* n=5 PPID cases receiving no treatment *Treatment Group:* Treatment group 1: n=20 PPID cases administered oral pergolide, 2 µg/kg once daily Treatment group 2: n=7 PPID cases administered oral cyproheptadine, 1.2 mg/kg once daily
Outcome Measures:	Clinical signs and endocrine testing
Main Findings:	Clinical improvement was reported more often with pergolide treatment compared with cyproheptadine. Normal ODST and TRH stimulation results were found in more horses treated with pergolide (35%; n=7/20) than in horses treated with cyproheptadine (14%; n=1/7) or no treatment (20%; n=1/5) ($p<0.05$). Mean concentrations for ACTH and insulin decreased significantly after 6-12 months of pergolide treatment ($p<0.05$) in contrast to a lack of significant changes for cyproheptadine and no treatment groups. There was no significant difference between cyproheptadine-treated and untreated horses in proportion of cases with normalisation of ODST and TRH. Decreased appetite was seen in some pergolide-treated horses during the first week of treatment, but resolved with a transient reduction in dose.
Interpretation of Results:	No information on case selection reported therefore unable to assess selection bias. No information provided regarding case selection for treatment, and allocation to treatment group was not randomised. There was substantial drop-out from the trial (58% of enrolled animals failed to complete the trial), which was unaccounted for and may bias the results towards positive outcomes. Results show that pergolide, at a dose rate of 0.002 mg/kg/day, was superior to cyproheptadine in alleviating clinical signs and improving endocrine test results and that side-effects were mild and transient.

Author, year: **Type of publication:**	Schott et al., 2014 Conference abstract
Study Design:	Retrospective cohort study
Aim/Objective of the Study:	To document long-term (>5 year) treatment response to pergolide in PPID cases.
Setting:	University referral hospital
Study Population:	n=30 PPID cases; diagnosed based on ODST or elevated ACTH (>50 pg/mL) during 2008-2009. Follow-up evaluations undertaken at 6 months, 2.5, 3, 3.5, 4.5 and 5.5 years after starting pergolide treatment.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=30 PPID cases administered oral pergolide, 2 µg/kg once daily. Pergolide dose was increased to 4 µg/kg/day when endocrine test results remained abnormal.
Outcome Measures:	Clinical signs and endocrine testing
Main Findings:	40% of cases survived >5 years (47% were euthanased, of which 3/14 due to chronic laminitis and 13% died during the study). All owners of surviving cases (n=12) remained satisfied with clinical improvement, and 4/12 cases (33%) remained on the starting pergolide dose (2 µg/kg once daily). At last follow-up, ACTH concentration was normal (<50 pg/mL) in 71% of cases and ODST results remained normal in 61% of cases.
Interpretation of Results:	No information on case selection reported therefore unable to assess selection bias. Small sample size, and based on referral hospital population. Did not appear to use seasonally adjusted reference interval for ACTH. Long-term treatment with pergolide resulted in clinical improvement, normalisation of endocrine test results, and owner satisfaction in a high percentage of cases.

Author, year: **Type of publication:**	Sgorbini et al., 2004 Journal publication
Study Design:	Case series
Aim/Objective of the Study:	To describe clinical presentation and outcome following treatment in 2 PPID cases.
Setting:	University hospital (assumed not stated)
Study Population:	n=2 PPID cases; diagnosed based on elevated basal ACTH, plus ACTH stimulation test measuring cortisol in case 1; follow-up over 14 months
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=2 Oral pergolide mesylate (Nopar® tablets), initial dose of 0.5 mg total dose every twenty-four hours, gradually increased by 0.5 mg every three days to a final total dosage of 3 mg once daily. Dose increased to 4 mg/day after 1 month in case 2 due to lack of response to treatment.

Outcome Measures:	Clinical signs and ACTH
Main Findings:	• Case 1: 18 year old Italian saddle horse mare. History of chronic recurrent laminitis, infertility, progressive hypertrichosis, hyperhidrosis, pendulous abdomen, lethargy, polyuria, polydipsia, and anorexia. Presented with a fever and increased respiratory rate. Blood work showed hyperglycemia, hyposthenuria, hyperinsulinemia, and elevated plasma ACTH. Clinical signs resolved and ovarian activity resumed with 6 to 7 weeks of pergolide treatment. ACTH dropped from 155 pg/mL to 26.8 pg/mL after 45 days and 27.7 pg/mL after 14 months of treatment. • Case 2: 25 year old French saddle horse mare with recurrent laminitis, hypertrichosis, pendulous abdomen, bulging supraorbital fat pads, severe polyuria and polydipsia, and a plasma ACTH of 370 pg/mL. There was no response to 3 mg of pergolide daily for one month. The dose was increased to 4 mg total dose per day and clinical signs were resolved with 6 weeks. ACTH dropped to 91.9 pg/mL at 2 months and to 59.1 pg/mL after 4 months of treatment. Polyuria and polydipsia significantly improved but did not completely resolve.
Interpretation of Results:	No information on case selection reported therefore unable to assess selection bias. Small sample size from unknown population, with both showing advanced clinical signs of PPID. Pergolide doses were 3 and 4 mg for each case respectively. Improvement in clinical signs, including a return of cyclic activity in one mare where this had been a problem, and ACTH (normalisation for one case and improvement by >50% in the other) within 6-8 weeks of pergolide treatment. No adverse events observed after 14 months of treatment.

Author, year: **Type of publication:**	Spelta and Axon 2012 Journal publication
Study Design:	Case series
Aim/Objective of the Study:	To describe clinical signs exhibited by PPID cases in the hot, humid conditions of a tropical climate and their response to treatment.
Setting:	First opinion practice
Study Population:	n=11 PPID cases (23 diagnosed with PPID from 2002-2010; however 12 cases were not available for follow-up). Cases comprised 4 light horse breeds and 7 ponies, age range 9 – 30 years; 5 mares and 6 geldings. PPID diagnosed based on ODST and clinical signs in 8 cases; remaining 3 cases diagnosed on presence of overt hypertrichosis only.
Intervention Investigated:	*Control Group:* No treatment (n=4)
	Treatment Group: n=7 PPID cases treated with oral pergolide mesylate (Permax®) at an initial dosage of 0.001 mg/kg/day with incremental increases of 0.001 mg/kg/day every 3-8 weeks until clinical resolution was achieved.

Outcome Measures:	Improvement of clinical signs and ODST (if treated). Clinical stabilisation defined as resolution of all clinical signs and included an improvement of lameness to an Obel laminitis grade 0–1, with no requirement for non-steroidal anti-inflammatory drugs; and active hair coat shedding in spring or appropriate hair coat growth in the winter while on treatment.
Main Findings:	Improvement of clinical signs in all pergolide-treated horses within 8 – 43 weeks (however results table indicates clinical resolution in 2 – 27 weeks, excluding the relapsing case). Resolution of clinical signs, including hair coat shedding, was longer (20-30 weeks) when treatment was started in autumn and winter (4 cases) than when treatment started in spring or summer (2-8 weeks; 3 cases). All 5 cases presenting with anhidrosis, heat stress and/or secondary exercise intolerance that were treated with pergolide had resolution of these clinical signs. One PPID case maintained on 0.014 mg/kg/day of pergolide failed to shed winter coat and had a recurrence of sinusitis after 14 months; therefore dose of pergolide was increased to 0.0023 mg/kg/day, which resulted in clinical resolution. One pony had a normal ODST result when treated with pergolide at 0.0015 mg/kg/day, but did not shed its coat, although hair coat shedding was achieved when the dose was increased to 0.0022 mg/kg/day. Unable to determine improvement in ODST results following pergolide treatment as text description of case numbers that had repeat ODSTs does not reconcile with data presented in results table. Four cases were managed with improved husbandry alone (untreated control group), which only resulted in weight stabilisation. There was no improvement in dorsum muscle atrophy, fat pad deposition, anhidrosis or heat stress with secondary exercise intolerance. Two untreated horses were euthanased because of severe heat stress and associated secondary exercise intolerance during the summer following diagnosis.
Interpretation of Results:	No information on case selection reported therefore unable to assess selection bias. Small sample size. No information provided regarding nature and duration of follow-up period (presumed to be based on clinical records). 3 of 11 cases lost to follow-up after 2-5 years, and outcome for other cases reported from 1-7 years after diagnosis. Systematic bias in that 3 of the 4 untreated horses did not have a diagnostic test and had presumptive diagnosis based on overt hypertrichosis. No adverse events observed with pergolide treatment. Provides low quality supportive evidence for the efficacy of pergolide.

Author, year: **Type of publication:**	Walsh et al., 2009 Journal publication
Study Design:	Case series
Aim/Objective of the Study:	To determine whether insulin concentration is correlated with severity of clinical laminitis in horses with EMS or PPID and to

	determine how rapidly pergolide treatment reduces circulating ACTH in horses with PPID.
Setting:	First opinion practice
Study Population:	25 horses were included from December 2004 to May 2007 and assigned to one of three groups: n=6 PPID; diagnosed based on basal plasma ACTH >70 pg/mL (laboratory reference interval 9-35 pg/mL); mean age 28.5 ± 8.14 years n=10 EMS; diagnosed based on normal ACTH and plasma insulin >70 mIU/mL (laboratory reference interval 10-30 mlU/mL); mean age 15.5 ± 5.11 years n=9 controls; with no history of laminitis and plasma ACTH and insulin within normal reference intervals; mean age 12 ± 7.6 years Blood samples were collected at an initial visit, and then at regular intervals for the next 12 months. Plasma values for ACTH, cortisol and insulin and serum values for glucose and total thyroxine (T4) were obtained. Follow-up 2.5 years
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=6 PPID cases (4 horses; 2 ponies); oral pergolide, 1 mg orally daily The 2 ponies in the PPID group died after 2 months, and a complete dataset was available for only 3 pergolide-treated horses.
Outcome Measures:	Clinical signs and ACTH concentration
Main Findings:	After treatment with pergolide, mean ACTH concentration of the PPID group decreased within one week from >120 pg/mL to ~ 70 pg/mL where it remained for the rest of the study, except the autumn where a seasonal peak was observed. Mean baseline plasma insulin concentration was not different between EMS (53.0 ± 16.3 mlU/mL) and PPID cases (52.0 ± 21.6 mlU/mL). Including both EMS and PPID cases, there was a significant correlation between laminitis Obel grade and baseline plasma insulin concentration.
Interpretation of Results:	Small sample size. Data regarding number of cases meeting inclusion criteria but not enrolled in study are not reported, therefore unable to assess selection bias. Results of this field-based study showed improvement in PPID cases treated with pergolide (1 mg orally daily), with ACTH decreasing within 1 week of commencing treatment; however, complete data were available for only 3 pergolide-treated horses. Limited data available on improvement of clinical signs other than laminitis. Statistical significance for decrease in ACTH was unlikely to be achieved due to the low power of the study (due to lack of numbers and high variation of plasma ACTH concentration), but the raw data represented graphically showed a marked decrease.

Author, year: **Type of publication:**	Watson et al., 1998 Conference abstract

Study Design:	Case series
Aim/Objective of the Study:	To evaluate the effect of long-term treatment with oral pergolide in PPID cases.
Setting:	University referral hospital
Study Population:	n=6 PPID cases; diagnosed based on ODST was used to evaluate PPID and treatment efficacy.
Intervention Investigated:	*Control Group:* Not applicable
	Treatment Group: n=6 PPID cases treated with oral pergolide mesylate at 1.8 to 2.8 µg/kg/day for 6 to 26 months
Outcome Measures:	Clinical signs and ODST. Pituitary gland mass was evaluated in two horses: one by magnetic resonance imaging and one at post mortem.
Main Findings:	All 6 horses responded to pergolide treatment, as measured by improvement in clinical signs and ODST, without a need to increase the dose of pergolide over the follow-up period. Improvements in clinical signs were seen before improvement in ODST in 2 of the 6 cases. No side effects of treatment were observed.
Interpretation of Results:	Very limited information provided in conference abstract. No information on case selection reported therefore unable to assess selection bias. Small sample size. Reported that pergolide can be effective for extended periods (>2 years) at these doses without a need to increase the dose. No side effects reported. Clinical signs appeared to improve before normalisation of ODST results.

Author, year: **Type of publication:**	Williams 1995 Conference abstract
Study Design:	Case control study
Aim/Objective of the Study:	To describe clinical presentation and outcome following treatment in 14 PPID cases.
Setting:	Private practice (first opinion and referral hospital; however not stated as to whether cases recruited from first opinion, referral or both)
Study Population:	n=23 PPID cases (24 in title); diagnosis of PPID by TRH stimulation and clinical signs. Treated for up to 3.5 years
Intervention Investigated:	*Control Group:* n=9 untreated PPID cases
	Treatment Group: n=14 treated with one or more of: Cyproheptadine (Periactin), dose not specified Low dose oral pergolide (Celance® tablets) maximum of 1 to 2 mg total dose per day High dose oral pergolide (Celance® tablets) maximum of 4 to 5 mg total dose per day
Outcome Measures:	Treatment response was evaluated by resolution of clinical signs and return to normal serum glucose concentration. In horses treated for a significant period of time, TRH stimulation tests and cortisol levels were monitored.

Main Findings:	Four of the 9 untreated animals were euthanased due to clinical signs of PPID within 6 months. Cyproheptadine caused little improvement in clinical signs and the cyproheptadine-treated cases that survived were changed to pergolide treatment. High-dose pergolide caused immediate clinical improvement, but the cost of this regimen was prohibitive. The clinical response to low-dose pergolide was encouraging and was more economical.
Interpretation of Results:	Very limited information provided in conference abstract for poster presentation. No information on case selection reported therefore unable to assess selection bias. Small sample size. Selection criteria for the 3 treatment options not reported, and treatment allocation was not randomised. Results of this study show a lack of efficacy of cyproheptadine, with no horses in this group responding, but all animals treated on the high dose pergolide showed an excellent and repeatable response. The author concludes positively about the low dose regimen as an alternative.

Appendix 5: Summary of studies presenting data regarding the efficacy of pergolide to improve clinical signs of PPID and ACTH levels included in a systematic review of published literature

Reference	Study design	Country	Sample size	Age of PPID cases	Selection of study population	PPID case definition	Method of outcome data collection	Main limitations
Aleman et al., (2006)	Case control study	USA	15 PPID cases, of which 3 treated with pergolide (plus 16 normal age matched controls)	Median age 21 years (range 15 – 28 years)	Non-random selection – university research herd and university hospital cases; details on recruitment of horse owners/selection of horses not reported	Clinical signs and ODST and/or serum ACTH concentration (diagnostic cut-off for ACTH for inclusion as PPID case not reported although reference interval of 2 – 10 pmol/L (4.5-45.5 pg/ml) reported with respect to assessing response to treatment).	Laboratory endocrine assays and muscle biopsy	▪ Sample selection likely to introduce bias ▪ Laboratory tests poorly described ▪ Small sample treated with pergolide ▪ Selection criteria and baseline details for treatment unknown (12 of 15 PPID cases not treated due to financial constraints) ▪ Unable to determine if seasonality accounted for during recruitment
Anon (2011a)	Uncontrolled, non-randomised field trial	USA	122 PPID cases	Age range 10 – 35 years	Non-random selection of hospital or client owned horses – details on recruitment of horse owners not reported. PPID cases enrolled November – January to avoid baseline	A hypertrichosis score >1/3 and either a plasma ACTH concentration >50 pg/mL or a positive ODST test	Objectively scored clinical examination and laboratory endocrine assays	• Unable to determine if sample selection could introduce bias

					endocrine tests during autumn months			
Beech et al., (2002)	Uncontrolled, non-randomised field trial	USA	14 PPID cases, of which 9 treated with pergolide	≥20 years (n=11) ≤19 years (n=3)	Non-random selection – details on recruitment of horse owners not reported	Clinical signs and plasma ACTH (n=12) (diagnostic cut-off for ACTH for inclusion as PPID case not reported) and/or ODST (n=4)	Clinical examination and laboratory endocrine assays	• Unable to determine if sample selection could introduce bias • Small sample treated with pergolide • Study design complicated by prior treatment with Vitex agnus castus • Unable to determine if seasonality accounted for during recruitment
Beech et al., (2009)	Non-randomised controlled trial	USA	15 PPID cases, of which 8 treated with pergolide (plus n=27 healthy controls)	Mean age 21.3 ± 4.3 years	No information provided regarding study population selection	At least 2 "classical clinical signs" of PPID	Laboratory endocrine assays	• Unable to determine if sample selection could introduce bias • Small sample size • Diagnostic criteria main outcome versus response to treatment
Christen et al., (2018)	Randomised placebo-controlled trial	Switzerland	24 pre-clinical PPID cases, of which 10 randomly allocated	Mean age 24.8 ± 3.6 years (range 14 – 32 years)	Non-random selection – cases identified by screening of horses at a foundation for retired equids.	ACTH >35pg/mL in July. No obvious clinical signs – defined as pre-clinical PPID	Laboratory endocrine assays	• Sample selection likely to introduce bias • Small number of treated cases and high loss to follow-up for a short study

			to pergolide treatment group (however 5 lost to follow-up) (plus 24 healthy controls with normal ACTH)					• Does not state whether study was blinded
Donaldson et al., (2002)	Case series	USA	27 PPID cases, of which 20 treated with pergolide	Median age 19 years (range 4 –29 years)	Records of horses/ponies treated at one university's ambulatory service between June 1996 and November 2001.	Plasma ACTH >50 pg/mL, at least 1 clinical sign of PPID, and evaluation before and after treatment	Clinical examination and laboratory endocrine assays	• Single practice population • Inherent biases due to retrospective study design - potential for introduced biases/errors through data collection via clinical records • Selection criteria for treatment unknown • Seasonality not accounted for during recruitment
Dunkel et al., (2014)	Case series	UK and USA	6 PPID cases	Age range 17 – 28 years	Non-random selection – cases identified via hospital records	Clinical signs, and plasma ACTH (using varied reference intervals, seasonally adjusted	Clinical examination and laboratory endocrine assays	• Sample selection likely to introduce bias due to retrospective study design, hospital population and

						in 3 cases) (n=4) or ODST (n=2)		concurrent disease - potential biases/errors through data collection via clinical records • Descriptive observational reports • Seasonality not always accounted for during recruitment
Froin et al., (1998)	Case series	Germany	5 PPID cases	Not reported	No information provided regarding study population selection	Not reported; presumed to be based on elevated ACTH	Clinical examination and laboratory endocrine assays	• Abstract only; insufficient information reported to assess risk of bias • Small sample size • Unable to determine if seasonality accounted for during recruitment
Gehlen et al., (2014)	Case control study	Germany	38 PPID cases, of which 13 treated with pergolide	Mean age 24 years (range 16 – 38 years)	No information provided regarding study population selection	ACTH ≥50pg/ml in January plus advanced clinical signs of PPID	Laboratory endocrine assays	• Unable to determine if sample selection could introduce bias • Small sample treated with pergolide • Single time point comparison between treated and untreated PPID animals therefore effect of treatment not directly measured • Selection criteria for treatment unknown

								• Seasonality not accounted for during recruitment
Horn et al., (2019)	Retrospective cohort study	Australia	274 PPID cases, with 197 of these cases having follow-up data. 218 of PPID cases treated with pergolide; number of pergolide-treated cases with complete follow-up data not reported	Median age at PPID diagnosis 21 years (range 8 – 42 years)	Retrospective review of clinical records of 8 different veterinary institutions (57% of cases were first opinion and the remaining 43% were referral cases)	ACTH (n=263 cases) cut-off values adjusted by season and location: autumn cut-off range 77.4 – 101 pg/mL and non-autumn cut-off range 29.7 – 67 pg/mL And/or positive ODST (n=9) or TRH stimulation test (measuring ACTH; n=12) plus 2 cases diagnosed at post mortem examination	Clinical examination and laboratory endocrine assays	• Inherent biases due to retrospective study design - potential for introduced biases/errors through data collection via clinical records • Loss to follow-up and missing data • The cited seasonally adjusted ACTH cut-off values utilised for PPID case definition were not available for the first 10 years of the study period, and may therefore differ from diagnostic cut-off values utilised by treating veterinary surgeons at the time of case presentation
Innerå et al., (2013)	Uncontrolled, non-randomised field trial	USA	8 PPID cases (plus 4 aged matched controls)	Mean age 24 years (range 20 – 29 years)	Non-random selection – recruitment of horses and owners via university hospital	Characteristic excessively long hair coats and ODST results (controls had normal coat shedding and	Clinical examination including scoring of hypertrichosis, skin punch biopsy	• Sample selection likely to introduce bias • Small sample size • Referral hospital population

						normal ODST results)	samples and laboratory endocrine assays	
Love (1993)	Case series	UK	5 PPID cases, of which 1 treated with pergolide	Age range 13 – 34 years	Non-random selection – cases referred to a university hospital	TRH stimulation test (measuring cortisol)	Clinical examination and laboratory endocrine assays	• Descriptive observational reports • Referral hospital population
McFarlane et al., (2017)	Uncontrolled, non-randomised clinical trial	USA	6 PPID cases	Mean age 24.3yrs (range 18 – 28 years)	Non-random selection – University research herd	Clinical signs and TRH stimulation test measuring ACTH	Laboratory endocrine assays	• Sample selection likely to introduce bias • Small sample size • Research herd population • No control/comparison
Orth et al., (1982)	Case series	USA	2 PPID cases, one treated with dopamine agonists (10 normal controls)	7 and 12 years	Non-random selection	Clinical signs	Laboratory endocrine assays	• Multiple experimental interventions on single horse • Short term effect of dopamine agonists (up to 48 hours)
Pease et al., (2011)	Case control study	USA	8 PPID cases, of which 6 treated with pergolide	Mean age 24 years (range 18 –28 years)	Non-random selection – animals donated to university hospital	Hypertrichosis and abnormal ODST results (controls had no hypertrichosis and	Clinical examination, laboratory endocrine assays and CT measurement	• Sample selection likely to introduce bias • Small sample size • Selection criteria for treatment unknown

			(3 aged controls)			normal ODST results)	of pituitary size	• Relevance to clinical practice limited
Perkins et al., (2002)	Retrospective cohort study	USA	39 PPID cases, of which 10 treated with pergolide	Not reported	Non-random selection – referring veterinary surgeons recruited via diagnostic laboratory	Samples submitted to a diagnostic laboratory with elevated ACTH result above upper limit of reference interval (>35 pg/mL)	Vet-reported survey data and laboratory endocrine assays	• Based on laboratory sample • Reliant on vet surveys • Response rate or drop out not reported • Seasonality not accounted for during recruitment
Peters et al., (1995)	Case series	USA	9 PPID cases	Age range 16 – 32 years	Non-random selection - horses presented for evaluation in private practice	Clinical signs and ODST	Laboratory endocrine assays and owner-reported observations	• Abstract only; information limited • Potential for introduced biases/errors from owner-reported data • Small sample size
Pongratz et al., (2010)	Retrospective cohort study	Switzerland	38 PPID cases	Mean age 22 years (range 13 – 36 years)	Non-random selection – owners recruited directly through university hospital or via their practice vet	Clinical examination, laboratory analyses including ODST (17/38) and/or ACTH (24/38) (reference intervals <50 pg/mL in horses and <30 pg/mL in ponies) and had been receiving pergolide for a minimum of one month	Owner questionnaire	• Sample selection may introduce bias • Variable follow-up periods • Owner-reported data - potential for introduced biases/errors • Seasonality not accounted for during recruitment

Rendle et al., (2013)	Retrospective cohort study	UK	2,122 PPID cases	Not reported	Retrospective review of submissions to a hospital laboratory	Laboratory diagnosed PPID cases in which ACTH concentration was measured before and after pergolide treatment	Laboratory endocrine assay results obtained from clinical records	• Retrospective study design - potential for introduced biases/errors through data collection via laboratory records • Abstract; information limited • Treatment duration not reported
Rendle et al., (2018)	Retrospective cohort study	UK	19 PPID cases	Not reported	Retrospective review of clinical records	Clinical signs and ACTH >50 pg/mL in July or >100 pg/mL in August – October	Laboratory endocrine assay results and clinical signs data obtained from clinical records	• Retrospective study design - potential for introduced biases/errors through data collection via clinical records • Abstract; information limited • Not possible to fully determine clinical improvement from data reported
Rohrbach et al., (2012)	Retrospective cohort study	USA	44 PPID cases (follow-up available for 34/44)	Mean age 21.6 ± 6.6 years	Retrospective clinical record search plus horses assessed at a university hospital – convenience sample followed up	Database search using PPID or synonym of. Records had to include clinical signs or laboratory result for inclusion in follow-up.	Retrospective review of clinical records and owner questionnaire	• Sample selection likely to introduce bias • Referral hospital population • Small sample treated with pergolide • Significant loss of follow-up and potential for bias/errors from owner-reported data

								• Unable to determine if seasonality accounted for during recruitment
Schott et al., (2001)	Uncontrolled, non-randomised field trial	USA	32 PPID cases	Median age 23 years (range 12 –34 years)	Non-random selection – horses presented for evaluation at a university hospital or with Michigan veterinarians in field practice	Clinical signs and positive ODST or TRH stimulation test (measuring cortisol)	Clinical examination and laboratory endocrine assays	• Sample selection may introduce bias • Treatment groups not randomised • High loss to follow-up which may bias toward positive results
Schott et al., (2014)	Retrospective cohort study	USA	30 PPID cases	Not reported	Details of case selection not reported	Clinical signs of PPID and supportive ODST results or elevated plasma ACTH (>50 pg/mL)	Laboratory endocrine assays and owner satisfaction	• Abstract only; insufficient information reported to assess risk of bias • Unable to determine if seasonality accounted for during recruitment
Sgorbini et al., (2004)	Case series	Italy	2 PPID cases	18 and 25 years	Details of case selection not reported	Clinical signs, elevated basal ACTH (>35 pg/ml), plus ACTH stimulation test measuring cortisol in one case	Clinical examination and laboratory endocrine assays	• Descriptive observational reports • Seasonality not accounted for during recruitment
Spelta and Axon (2012)	Case series	Australia	11 PPID cases, of which 7 treated with pergolide	Age range 9 – 30 years	Non-random selection - horses diagnoses at a first opinion practice and available for follow-up	Clinical signs and positive ODST (*n*=8) or hypertrichosis (*n*=3)	Clinical examination and laboratory endocrine assays (improvement in ODST in	• Sample selection likely to introduce bias • Follow-up time frame unclear

							pergolide-treated cases)	
Walsh et al., (2008)	Case series	USA	6 PPID cases	Mean age 28.5 ± 8.14 years	Non-random selection - horses diagnoses with laminitis/history of laminitis and PPID at a first opinion practice	Clinical findings and initial diagnostic tests (ACTH >70 pg/ml)	Clinical signs and ACTH concentration	• Sample selection likely to introduce bias • Small sample size and concurrent disease • Seasonality not accounted for during recruitment, ACTH concentration for inclusion relatively high
Watson et al., (1998)	Case series	USA	6 PPID cases	Not reported	Unclear how cases selected	ODST	Clinical signs and laboratory endocrine assays	• Abstract only; insufficient information reported to assess risk of bias • Small sample size
Williams (1995)	Case control study	UK	23 or 24 PPID cases	Not reported	Non-random selection - horses diagnosed at a referral practice	Clinical signs and TRH stimulation test (cortisol measured post-TRH)	Clinical signs and laboratory endocrine assays	• Abstract only; insufficient information reported to assess risk of bias

ACTH = Adrenocorticotrophic hormone, ODST = Overnight dexamethasone suppression test, POMC = Proopiomelanocortin, TRH = Thyrotropin-releasing hormone, CT = Computed tomography.

Chapter 5

A cross-sectional study of horses diagnosed with pituitary pars intermedia dysfunction in the United Kingdom: Demographics, management practices and factors associated with quality of life

A cross-sectional study of horses diagnosed with pituitary pars intermedia dysfunction in the United Kingdom: Demographics, management practices and factors associated with quality of life

Summary

Little information is currently available regarding management of horses with pituitary pars intermedia dysfunction (PPID) following diagnosis. This study aimed to evaluate the current management and treatment practices undertaken by owners and how these impact owner-perceived quality of life (QoL). Owners of horses diagnosed with PPID were recruited via a single equine veterinary practice and online to participate in a cross-sectional questionnaire study. The questionnaire included questions pertaining to management practices, preventive health care and general health as well as clinical signs of, and treatment for, PPID. Visual analogue scale questions were used to gather owner-perceived ratings of QoL on a scale of 1 (could not be worst) – 10 (could not be better).

A total of 377 useable questionnaires were returned. Horses were most frequently kept at livery yards (36.4%); the majority received pasture turnout (81.6%), daily forage (95.2%) and bucket/concentrate feeds (94.6%). Owner decisions regarding diet were predominantly based on personal experience (67.2%). Most owners reported restricting their horse's access to grazing for at least some period during the year (75.7%) and those with a history of laminitis spent fewer hours turned out year-round ($p=0.003$). Owners reported a median of 2 routine veterinary visits within the previous year and 56.8% of horses were reported to have ≥1 concurrent health condition. Median current QoL was 9/10; significantly higher than QoL at the time of diagnosis (median = 6/10; IQR 4.75-8) ($p<0.001$). Most horses were currently treated with pergolide (86.9%) which had a higher efficacy rating (median = 8/10; IQR 7-10) compared to complementary or alternative treatments (median = 5/10; IQR 2.5-7) ($p<0.001$). Pergolide treatment was associated with an improvement in QoL rating since the time of diagnosis ($p=0.002$) and pergolide treatment alone was associated with a higher QoL rating compared to treatment combinations or no treatment ($p=0.008$). Clinical signs associated with lower QoL ratings were laminitis ($p<0.001$), a curly/over-grown coat ($p=0.007$), lethargy/poor performance and patchy/excessive sweating (both $p=0.002$). Owners perceived their horses to have a very good QoL, with significant improvement reported since PPID diagnosis and pergolide treatment was positively associated with QoL

rating. Evaluation of QoL may be beneficial as a component of routine monitoring of PPID cases, and the impact of PPID on daily management should be considered alongside clinical factors to maximise QoL in horses diagnosed with PPID.

Introduction

Over a quarter (25%) of the equine population in the United Kingdom now consists of aged horses (≥15 years) (Mellor et al., 1999, 2001; Ireland et al., 2011a). Pituitary pars intermedia dysfunction (PPID) is a progressive neurodegenerative endocrine disorder of older equids (van der Kolk, 1997; McFarlane and Cribb, 2005; McFarlane et al., 2005b) which is prevalent and frequently diagnosed in older horses (McGowan et al., 2013a; Ireland and McGowan 2018). Clinical signs are varied and include hypertrichosis, muscle wastage and lethargy, and associated comorbidities such as laminitis (Schott, 2002; McFarlane, 2014). PPID can affect horses for a significant proportion of their life-time, with the median life expectancy following diagnosis reported to be nearly 10 years (Welsh et al., 2016). Effective management of the disease is vital to maximise quality of life (QoL) following diagnosis.

There is currently little information available regarding how horses with PPID are managed by their owners including the routine health care and treatment they receive. Previous studies have focused on the management and care of the general equine geriatric population as a whole with significant changes in diet, exercise and veterinary care reported as horses age (McGowan et al., 2010a; Ireland et al., 2011a, 2011b). Pergolide is the treatment of choice for PPID and has been shown to be effective at managing clinical signs (Anon, 2011a; Chapter 4). However, although veterinary surgeons recommend treatment, owners are responsible for the day to day decisions regarding management and treatment (Ireland et al., 2011a,b) which are central to controlling PPID. Little is currently known about owner compliance with veterinary recommended treatment or owner perception of response to PPID treatment. In human medicine, interventions such as structured self-assessments can improve patient management of chronic conditions producing positive outcomes including better monitoring, increased uptake of treatment and fewer clinical signs allowing patients to enjoy a good QoL (Skevington et al., 2001; Lorig et al., 2013; Reynolds et al., 2018).

The term QoL is defined by the World Health Organisation (WHO) as a 'state of complete physical, mental and social well-being, and not merely the absence of disease and infirmity' (WHO, 1997). In veterinary medicine, definitions of QoL vary. QOL is generally considered to be closely linked to welfare but encompasses the broader concepts of physical and mental health, positive and negative feelings and the ability to exhibit natural behaviours (Clark, 1997; McMillan, 2003; Wojciechowska and Hewson, 2005; Broom, 2007). Owners of aged horses perceive QoL to be an important health issue (McGowan et al., 2010b) and are likely to be the ones best placed to determine the QoL of their animal (McMillan, 2000; Wojciechowska and Hewson, 2005; Ireland et al., 2011c). In small animal medicine, tools have been developed to evaluate QoL generally (Yeates and Main, 2009; Lavan, 2013; Mullan, 2015) and in animals with chronic conditions (Wiseman-Orr et al., 2004; Freeman et al., 2012). In equine medicine, owner-perceived QoL in geriatric horses has previously been investigated, with owners reporting that horses enjoyed a good QoL (McGowan et al., 2010b; Ireland et al., 2011c) however, there is currently no information specifically regarding QoL in horses with a chronic condition such as PPID. As the most common endocrine disorder of older equids (McGowan et al., 2013a), PPID is becoming increasingly important in equine practice. An understanding of QoL in horses with PPID and factors that influence it could be used to facilitate a more individual animal approach to care, improve disease monitoring and aid clinical decision making (Bradley, 2001; Yeates and Main, 2009; Mullan, 2015).

This study aimed to evaluate the current management practices owners undertake when caring for horses with PPID, including health care and treatment, and how these impact owner-perceived QoL.

Materials and methods

Selection of study sample

A cross-sectional questionnaire was distributed to owners of horses and ponies diagnosed with PPID. Two routes were used to recruit eligible owners; firstly, a review of the University of Liverpool Equine Practice (UoLEP) database was undertaken to identify PPID cases and secondly appropriate social media platforms were also used to recruit eligible owners. For PPID cases recruited through the UoLEP, diagnosis was confirmed by clinical record of a basal plasma ACTH concentration above the upper limit of seasonally adjusted reference

intervals (>29.7 pg/ml November-July or >47 pg/ml August-October). For those recruited online, PPID diagnosis was determined by owner confirmation of a positive diagnostic test result along with the reported reason for carrying out diagnostic testing, which included one or more of the following: age, veterinary surgeon suspected PPID, active laminitis, previous history of laminitis, abnormal fat distribution, pot-bellied appearance, muscle wastage, fat pads around the eyes, excessive/patchy sweating, increased drinking and urination, lethargy, recurrent infections, abnormal coat shedding, curly coat/overgrown coat and changes to coat colour/texture. Horses diagnosed prior to 1st January 2014 were excluded to reduce the risk of owner recall bias. All owners recruited via the UoLEP received a letter and information sheet (Appendices 1 and 2) informing them about the study, and to optimise recruitment they were contacted by the author via telephone to complete the questionnaire. For participants recruited online, the study information and a link to the questionnaire were made available through the UoLEP and Talk About Laminitis[1] Facebook pages (Appendix 3). The questionnaire link remained open for four weeks. This study was awarded institutional ethical approval on the 18th May 2018, reference number VREC667.

Questionnaire design

Effective management and treatment strategies have been identified as important areas for further investigation (Chapter 2). The questionnaire was therefore designed to gather data on current management factors such as feed, exercise, hoof care, general health, medical treatment, alternative treatments and owner-perceived QoL (Appendix 4). Additionally, information regarding management practices, such as stabling/turnout routine, throughout the year and any changes since PPID diagnosis in clinical signs, medical treatment/alternative treatments and QoL. The questionnaire was designed to facilitate completion over the phone or online using data capture software (KwikSurveys). The majority of the questions were closed-ended with predefined options provided for participants to indicate their response. Where "Other" was provided as an option, additional space was provided for respondents to provide further information or criteria. A number of open-ended questions were also used with boxes provided for free written text.

[1] Talk about laminitis (TAL) is an online forum and educational service established by Boehringer Ingelheim for owners of horses diagnosed with PPID. It aims to raise awareness of the link between laminitis and endocrine disease.

Questions around QoL were developed from visual analogue scales (VAS) utilised in human medicine (Katsura et al., 2003) and small animal practice (Belshaw et al., 2015). For these VAS questions, a number of statements and factors/measures were provided with Likert-style descriptors at the two extremes of the scale. The factors/measures were chosen to encompass activities of daily living (Ireland et al., 2011c) and the five freedoms (Mellor, 2016). Owners were asked to rate their horse's appetite, demeanour, overall QoL and QoL at time of PPID diagnosis on a scale of 1 (could not be worse) to 10 (could not better). Additionally, they were also asked to rate the level of discomfort they perceived they horse to be in from 1 (no discomfort) to 10 (severe discomfort). Owner-perceived efficacy of pergolide and alternative treatments, where applicable, were also assessed using a rating scale of 1 (not at all effective) to 10 (extremely effective). Owner-reported body condition score (BCS) was adapted from Carroll and Huntington (1988), with descriptions provided for each score (Appendix 4). Owners were requested to provide separate BCS ratings for their horse's neck (1-5 scale), ribs and pelvis (0-5 scale). Owners of more than one horse with PPID were asked to complete individual questionnaires for each horse.

Data Analysis

All data were exported into a Microsoft Excel spreadsheet, using the KwikSurveys software, and each horse was allocated a unique identification number. Statistical analyses were performed using commercial statistical software (IBM SPSS Statistics Version 25). For BCS, the mean of the three owner-reported scores (neck, ribs, and pelvis) was calculated to provide an overall BCS. Continuous data from the questionnaire responses were not normally distributed and are therefore described as medians with interquartile ranges (IQR) and categorical data are described as proportions. Kruskal-Wallis, Mann-Whitney U and Wilcoxon signed-rank tests were used to test differences in median values of continuous variables between categories of categorical variables. Where appropriate, Pearson Chi-squared or Fisher's exact tests were used to assess associations between categorical variables. Current overall QoL was assessed using raw interval data from the responses to the question 'how would you rate your horses current overall QoL?' where respondents provided a rating on a scale of 1-10, as well as using these data to derive a categorical outcome, where a rating of ≤4/10 was defined as a poor QoL, 5-7/10 as average and ≥8/10 as a good QoL. Statistical significance was set at $p<0.05$. For a number of questions there was a low level of item omission and some questions were only answerable conditionally on

other responses, therefore the denominators for the results vary, and are reported throughout.

Results

Case demographics

In total, 377 questionnaires met inclusion criteria with a median of one horse per owner (IQR 1-2). Questionnaires were completed between July 2018 and February 2019 with the majority being completed online in March and April 2019 (80.6%; n=304/377). The response rate of owners registered with the UoLEP was 70.2% (n=73/104). Horses had been diagnosed with PPID between 1st January 2014 and 31st March 2019. The median age of the study population was 23 years (IQR 19-26 years; range 7-40 years) and the median duration of ownership was 12 years (IQR 7-18 years; range 0.3-35 years). The population comprised 54.5% (n=205/376) geldings, 44.9% (n=169/376) mares and 0.5% (n=2/376) stallions. Ponies (≤147.3 cm in height) made up 52.1% (n=195/374) of the study population, and the most numerous breeds were UK and Irish Native/native-cross breeds (46.9%; n=176/375), followed by Thoroughbred/Thoroughbred-cross (11.7%; n=44/375) and Irish Draught/Irish Draught-cross (10.4%; n=39/375).

Management

Horses were mainly kept at livery yards (36.4%; n=137/376) or at the owner's private premises (30.3%; n=114/376). Most owners reported that their horse spent at least some time in an outside turnout area: median turnout time throughout the year is shown in Figure 1. Turnout was generally at pasture (81.6%; n=271/332) and most commonly in a paddock <1 acre in size (43.4%; n=145/334) with at least one equine companion (75.0%; n=252/336). Horses spent a median of 20 hours (IQR 12-24) turned out on pasture in the summer and 8 hours (IQR 6-8) in the winter. Compared to horses without a history of laminitis, horses with a history of laminitis spent fewer hours turned out throughout the year overall (p=0.003) (median hours turned out 15 and 9.5, respectively). Furthermore, the duration of daily turnout for animals with a history of laminitis was significantly shorter throughout each season compared to horses without a history of laminitis; during the spring (p=0.009), summer (p=0.004), autumn (p=0.005) and winter months (p=0.02) (Figure 1). The majority of owners restricted their horse's access to pasture for a period of time

throughout the year (75.7%; n=253/334), and the most frequently used methods were strip grazing (25.4%; n=85/334), time restricted grazing (17.1%; n=57/334) and use of a bare/dirt paddock 13.5%; n=45/334). Only 24.0% (n=79/328) of horses were turned out at pasture for 24 hours per day all year round. Most of the study population received daily supplementary forage (95.2%; n=318/334), which was most frequently fed on an ad-lib basis (34.3%; n=109/318). The majority also received some form of bucket/concentrate feed (94.6%; n=306/325). Owner feeding practices are shown in Table 1.

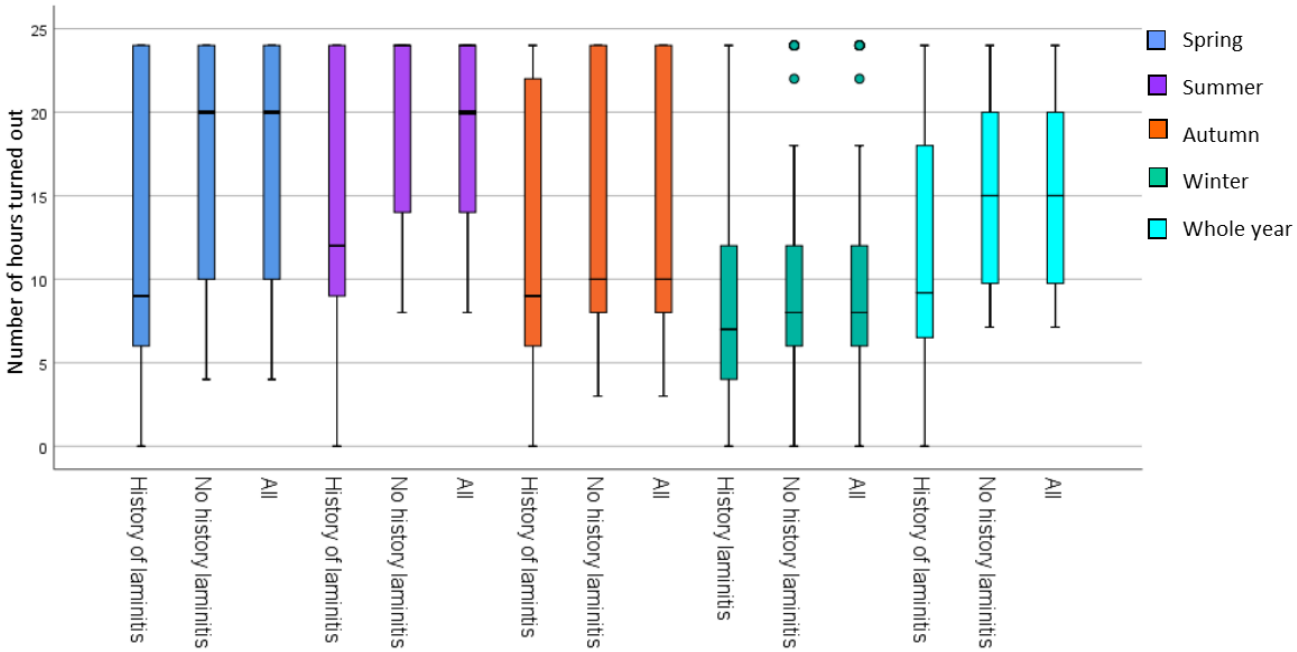


Figure 1: Box and whisker plot of owner-reported hours spent turned out during different seasons and throughout the year overall (median represented by horizontal lines within each box, which represents IQR) in a cross-section of horses diagnosed with PPID in the UK, including differences between horses reported to have a history of laminitis, horses with no reported history of laminitis and all included horses.

Table 1: Current owner-reported feeding practices for a cross-section of horses diagnosed with PPID in the UK

Feed type	N	%
Forage (n=318)		
Dry hay	158	49.7
Soaked hay	101	31.8
Haylage	81	25.5
Steamed hay	15	4.7
Hay replacer	11	3.5
Straw	8	2.5
Bucket/concentrate feed (n=306)		
Chaff/chop	197	64.4
Sugar/fibre beet	103	33.6
Balancer	103	33.6
Fibre/grass cubes	85	27.8
Mash feeds	44	14.4
Veteran mix/cubes	41	13.4
PPID/laminitis specific feeds	40	13.1
Coarse mix	17	5.6
Conditioning mix/cubes	14	4.6
Bran	8	2.6
Cereals	5	1.6

Owners generally designed their horse's diet to maintain their horse's current weight (70.6%; n=233/330). Decisions regarding diet were predominantly based on their own personal experience (67.2%; n=227/338) with only 25.7% of owners reporting that veterinary advice influenced their feeding practices (n=87/338). Horses were most frequently reported to be in average body condition, with an overall owner-reported BCS calculated as 2/5 (60.0%; n=201/335). The most frequently reported individual scores were; neck 1/5 (56.1%; n=188/335), ribs 3/5 (34.9%; n=117/335) and hind quarters 3/5 (58.8%; n=197/335). Basing decisions regarding diet on personal experience was

associated with overall BCS (p=0.02), however making decisions based on veterinary advice was not significantly associated with overall BCS (p=0.27). Most owners reported monitoring their horse's weight on a regular basis (74.3%; n=248/334), most frequently 'by eye' without any formal measure (43.7%; n=145/332) or by using a weigh tape (38.9%; n=129/332).

Half of respondents reported that their horse received some form of regular exercise (50.8%; n=169/333), which was mainly low intensity hacking/pleasure riding (91.7%; n=155/169). The age of horses reported to be currently in work (median 21 years) was significantly lower compared to horses not currently in work (median 25 years) (p<0.001).

Preventive health care and concurrent health problems

Over half of the study population were unshod (53.5%; n=175/327). Of the horses reported to be shod (46.5%; n=152/327), 24.3% (n=37/152) received some form of remedial shoeing. There was a significant association between laminitis and remedial shoeing: 35.3% of horses with current active laminitis had remedial shoes (n=6/17) compared to 10.0% of horses without laminitis at the time of questionnaire completion (n=31/310) (p=0.001).

Within the preceding 12 months, horses were reported to have received a median of 2 (IQR 1-3) routine veterinary visits and a median of 1 (IQR 0-2) non-routine veterinary visits. For those that received non-routine veterinary visits (48.5%; n=183/377), the most frequently reported reasons were non-specific disorders (such as viruses or weight loss) (31.1%; n=57/183), lameness (excluding laminitis) and laminitis (both 26.8%; n=49/183).

The majority of horses were currently vaccinated against tetanus (75.9%; n=286/377) and equine influenza (67.1%; n=253/377). Most owners also reported their horse had regular dental examinations with around half receiving yearly checks (54.8%; n=182/332) and 36.1% receiving six monthly checks (n=120/332). Dental care was provided by equine dental technicians (59.2%; n=199/336) or veterinary surgeons (40.8%; n=137/336). The vast majority of owners (96.2%; n=326/339) described following an anthelmintic administration regimen, which was most frequently based on the results of faecal worm egg counts (FWEC; 50.1%; n=170/339) and worming at the same time each year (26.3%; n=89/339).

Over half of horses were reported to currently have at least one concurrent health condition (56.8%; n=214/377), of which the most frequently reported were osteoarthritis (38.3%; n=82/214), equine metabolic syndrome (EMS) (24.8%; n=53/214) and respiratory disorders such as severe equine asthma (7.0%; n=15/214).

Diagnosis of PPID and clinical signs

The median time between diagnosis of PPID and completion of the questionnaire was 2 years (IQR 1-3.5 years; range 0.1-5 years). Median horse age at time of diagnosis was 20 years (IQR 17-24 years). The majority of horses were reportedly diagnosed using basal ACTH concentration (98.9%; n=370/374). Where owners reported ACTH test results at time of diagnosis, 44.2% (n=91/206) were ≤100 pg/ml, 40.8% (n=84/206) were between 101-500 pg/ml and 15% (n=31/206) were >500 pg/ml. As only 32.4% (n=122/377) of owners provided an exact date of PPID diagnosis, analysis of owner-reported ACTH concentrations for different seasons was not undertaken.

Owners reported a variety of reasons for suspecting their horse had PPID, prior to diagnostic testing, and several of these reasons were significantly associated with age at the time of diagnosis (Table 2). Prior to diagnosis, 62.3% n=235/377 of horses were reported to have no previous history of laminitis. A small proportion of horses (9.0%; n=34/377) were not reported by their owners to be showing any specific clinical signs prior to diagnosis, with age being the sole reason for suspecting PPID. At the time of questionnaire completion, owners reported currently observing at least one clinical sign of PPID in 73.9% (n=252/341) of horses, of which the most frequently reported were muscle wastage (26.7%; n=91/341), overgrown coat (23.2%; n=79/341) and abnormal coat shedding (18.2%; n=62/341).

Table 2: Owner-reported reasons for suspecting PPID and associations with age at the time of diagnosis in a cross-section of horses subsequently diagnosed with PPID in the UK (n=377; with age at diagnosis reported for n=376)

Reported reason for suspecting PPID	N	%	Median age (years) with clinical sign/factor reported (IQR)	Median age (years) with clinical sign/factor not reported (IQR)	P value[1]
Active laminitis	108	28.6	19.0 (14.5-21.9)	21.0 (18.0-24.0)	<0.001
Veterinary suggested	97	25.7	20.5 (17.0-24.0)	20.0 (17.0-24.0)	0.58
Overgrown coat	88	23.3	21.3 (18.1-24.4)	20.0 (17.0-23.9)	0.03
Abnormal coat shedding	86	22.8	20.6 (18.0-25.0)	20.0 (17.0-24.0)	0.14
Horse age	85	22.5	22.0 (22.0-26.0)	20.0 (16.1-23.0)	<0.001
Lethargy/poor performance	85	22.5	20.0 (15.0-22.0)	20.5 (17.0-24.0)	0.04
Muscle wastage	82	21.8	21.0 (16.8-24.0)	20.0 (17.0-24.0)	0.62
Fat pads around the eyes	67	17.8	19.0 (15.0-21.0)	20.5 (17.0-24.0)	0.005
Increased drinking/urination	67	17.8	19.0 (15.0-22.0)	20.5 (17.0-24.0)	0.01
Abnormal fat distribution	66	17.5	19.0 (15.0-22.0)	20.5 (17.0-24.0)	0.008
Pot-bellied appearance	62	16.4	20.0 (15.0-24.0)	20.0 (17.0-24.0)	0.34
Recurrent infections	60	15.9	20.5 (16.5-23.5)	20.0 (17.0-24.0)	0.67
History of laminitis	55	14.6	18.0 (13.0-23.0)	20.5 (17.0-24.0)	0.006
Excessive/patchy sweating	49	13.0	19.5 (16.8-21.0)	20.0 (17.0-24.0)	0.04
Abnormal coat texture/colour	44	11.7	20.0 (17.0-26.0)	20.0 (17.0-24.0)	0.78
Curly coat	38	10.1	21.5 (17.0-26.3)	20.0 (17.0-24.0)	0.04

[1]Mann-Whitney U Test p values for the difference in age at time of diagnosis for each reason reported.

Treatment

The majority of horses had received pergolide treatment at some point after PPID diagnosis (94.1%; n=352/374), while 86.9% (n=325/374) were currently receiving pergolide treatment. Seventeen percent (n=65/374) currently received some form of complementary or alternative treatment and of these, 70.8% (n=46/65) also received pergolide. The most

frequently reported complementary or alternative treatments used were magnesium (43.1%; n=28/65), herbal supplements/feeds marketed for horses with PPID (41.5%; n=27/65) and *Vitex agnus castus* (Chaste berry) (26.2%; n=17/65). When asked to report what influenced their decision regarding treatment for PPID, owners most frequently stated advice from their usual vet (81.4%; n=307/377), their horse's QoL (59.7%; n=225/377) and ACTH test results (52.0%; n=196/377).

The median current dose of pergolide received per horse was 1mg per day (range 0.25-6mg) and this was given once a day by the majority of owners (93.0%; n=305/328), where daily dose alternated the median daily dose was calculated. Unsurprisingly, dose was associated with height ($p<0.001$) with 42.3% (n=71/168) of ponies receiving <1mg and 88.1% (n=128/158) of horses receiving ≥1mg. Owners reported that pergolide dose had not changed within the preceding 12 months for 65.8% (n=219/333) of horses, had increased for 24.0% (n=80/333) and decreased for 10.2% (n=34/333). Where modifications to the dose of pergolide administered had been made, the majority of owners reported consulting their vet before making changes (78.1%; n=89/114).

When owners were asked to rate treatment efficacy, pergolide had a higher efficacy rating (median = 8/10; IQR 7-10) compared to complementary or alternative treatments (median = 5/10; IQR 2.5-7) ($p<0.001$). Of those who provided a reason for the pergolide efficacy rating given, the most frequently reported were; an improvement in demeanour/behaviour (26.7%; n=94/352), improvement in ACTH levels (17.0%; n=60/352) and laminitis perceived to be more controllable (12.8%; n=45/352).

Only 8.0% (n=30/374) of horses currently received no PPID treatment of any kind. The median age of this subset of horses was not significantly different from those receiving any kind of treatment ($p=0.98$). The majority (76.7%; n=23/30) were reported to be currently showing at least one clinical sign of PPID, the most frequently reported of which were muscle loss (26.7%; n=8/30), curly/overgrown coat (23.3%; n=7/30) and fat pads around the eyes (20.0%; n=6/30). This subset had a median current QoL rating of 8 (IQR 7.75-10), and this was reported to have remained the same (n=11) or improved (n=17) since diagnosis in all but two cases.

Side effects of pergolide treatment

Of those owners who stopped pergolide treatment, the most frequent reason provided for not currently treating their horse with pergolide was observed side effects (95.5%; n=21/22). Just over half of owners who treated their horse with pergolide reported observing side effects during the first two weeks of treatment (56.5%; n=199/352). The most frequently reported side effects during this period were loss of appetite (31.5%; n=111/352), lethargy/poor performance (21.0%; n=74/352) and diarrhoea/loose faeces (6.0%; n=21/352). These side effects were reported to continue after two weeks in 50.8% (n=101/199) of cases. Some owners (14.5%; n=51/352) reported observing new side effects after two weeks of treatment, 76.5% (n=39/51) of which were reportedly observed in horses that had no side effects during the first two weeks of treatment. The most frequently reported side effects after two weeks of treatment were loss of appetite (28.1%; n=99/352), lethargy/poor performance (18.2%; n=64/352) and erratic/unpredictable behaviour (7.4%; n=26/352). Pergolide dose was not significantly associated with side effects observed during the first two weeks of treatment (p=0.41).

Quality of life

Overall horses with PPID were perceived to have a very good current QoL (median rating = 9/10; IQR 8-10). This was significantly higher compared to overall QoL rating at time of diagnosis (median = 6/10; IQR 4.75-8) (p<0.001). Quality of life was reported to have improved since diagnosis in the majority of horses (73.7%; n=258/377), no change was reported in 16.6% (n=58/377) and only 9.0% (n=34/377) were reported to have a worse QoL. Median ratings for other current QoL measures were; appetite 10/10 (IQR 8-10), level of discomfort 2/10 (IQR 1-4) and demeanour 9/10 (IQR 8-10). The factors most frequently reported by owners to influence overall QoL rating positively were pergolide treatment (22.3%; n=84/377) and access to grazing (16.2%; n=61/377), while laminitis was most frequently reported to have a negative impact on QoL (13.5%; n=51/377).

Treatment with pergolide was associated with a higher current overall QoL rating compared other treatment groups (Table 3). Horses receiving alternative treatments, whether in combination with pergolide or not, had a significantly lower QoL rating compared to those not receiving alternative treatments (Table 3). Pergolide treatment was also associated with an improvement in QoL rating since the time of diagnosis (p=0.002), with 75.9%

(n=203/303) of owners reporting an improvement in horses treated with pergolide compared to 59.6% (n=28/47) of those that did not receive pergolide. Several clinical signs and concurrent conditions were associated with a lower overall QoL rating, which are reported in Table 3. Discomfort ratings were significantly higher in horses currently reported to be suffering from laminitis (median = 4/10; IQR 2-7) or lethargy/poor performance (median = 5.5/10; IQR 2-5.25) compared to horses without laminitis (median = 2/10; IQR 1-3) and without lethargy/poor performance (median = 2/10; IQR 1-4) ($p<0.001$ and 0.02, respectively). Management factors associated with owner rating of overall QoL included type of turnout, the method used to restrict grazing and overall BCS (Table 4). Neither ACTH concentration nor age were significantly associated with QoL rating.

Table 3: Owner-reported current treatment and currently observed clinical factors associated with owner-perceived quality of life in a cross-section of horses diagnosed with PPID in the UK

Factor associated with QoL	Category	N	%	Median QoL rating[2]	Interquartile range	P value[1]
Current treatment (n=374)	Pergolide only	279	74.6	9	8-10	0.008
	Alternatives[3]	19	5.1	8	7.75-9.25	
	Pergolide & alternatives	46	12.3	8	7-9	
	None	30	8	8	7.75-10	
Alternative treatment (n=374)	Yes	65	17.4	8	7-9	0.003
	No	309	82.6	9	8-10	
Current active laminitis (n=341)	Yes	68	19.9	8	6-9	<0.001
	No	273	80.1	9	8-10	
A curly/over-grown coat (n=341)	Yes	79	23.2	8	7-8	0.007
	No	262	76.8	9	8-10	
Muscle wastage (n=341)	Yes	91	26.7	8	7-9.5	<0.001
	No	250	73.3	9	8-10	
Lethargy/poor performance (n=341)	Yes	21	6.2	7	6-9	0.002
	No	320	93.8	9	8-10	
Patchy/excessive sweating (n=341)	Yes	25	7.3	8	6-9	0.002

	No	316	92.7	9	8-10	
Equine metabolic syndrome (n=377)	Yes	53	85.9	8	6.25-10	0.002
	No	324	14.1	9	8-10	
Severe equine asthma (n=377)	Yes	15	4	8	6-9	0.006
	No	362	96	9	8-10	

[1]Kruskal Wallis and Mann-Whitney U Test p values for the difference in owner-perceived quality of life median ratings.

[2]Overall current owner-perceived quality of life rating on a scale of 1 (could not be worse) – 10 (could not be better).

[3]Alternative treatment included magnesium, herbal supplements/feeds marketed for horses with PPID and Vitex agnus castus (Chaste berry).

Table 4: Owner-reported management factors associated with owner-perceived quality of life in a cross-section of horses diagnosed with PPID in the UK

Factor associated with QoL	Grouping Variable	N	%	% with good QoL rating (≥8/10)[2]	% with average QoL rating (5-7/10)	% with poor QoL rating (≤4/10)	P value[1]
Type of turnout (n=332)	Non-grass area	15	4.5	53.3	20.0	26.7	
	Grass paddock	271	81.6	84.0	12.3	3.7	0.007
	Bare/dirt paddock	46	13.8	75.6	20.0	4.4	
Restricting access to grazing	No	81	24.5	91.4	8.6	0.0	0.006
(n=330)	Yes	249	75.5	77.5	15.7	6.8	
Method of restricting grazing	None	81	24.5	91.4	8.6	0.0	0.02
(n=330)	Strip grazing	84	25.5	76.2	17.9	6.0	
	Time-restricted	55	16.6	81.8	10.9	7.3	
	Track system	30	9.1	86.7	10.0	3.3	
	Grazing muzzle	35	10.6	80.0	14.3	5.7	
	Bare/dirt paddock	45	13.6	66.7	22.2	11.1	
Overall body condition score	1 (poor)	27	8.1	57.7	30.8	11.5	0.02
(scale of 0-5, however no owners	2 (average)	201	60	83.9	12.6	3.5	
reported a BCS of 0 or 5) (n=335)	3 (good)	93	27.8	81.5	10.9	7.6	
	4 (overweight)	14	4.2	71.4	28.6	0.0	

[1]Chi-squared test p value used to assess associations between categorical variables.

[2]Overall owner-perceived quality of life rating comprised the owner response to the question 'How would you rate your horse's current overall quality of life?' on a scale of 1 (could not be worse) – 10 (could not be better).

Discussion

The results of this study provide a detailed description of owner practices regarding management, healthcare and treatment in horses diagnosed with PPID. The study has also provided important novel information regarding owner-perceived QoL and factors associated with that QoL rating in their horses.

The sample used in this study comprised horses diagnosed with PPID over a five-year period. Owners were recruited via a review the UoLEP database and online through appropriate social media channels. This was a convenient, efficient and cost-effective way of reaching a wide range of potential participants. As with all questionnaire-based research there is the risk of response bias and this method meant it was not possible to calculate an overall response rate to the questionnaire. However, the response rate from owners registered with the UoLEP was high. In this study, horse owners may have been more likely to respond if they had higher standards of care or were veterinary registered and following veterinary treatment recommendations. Comparison with the general target population is also not possible because there is currently no way of quantifying non-veterinary registered PPID cases. However, the field-based sample presented here is likely to be more representative of the veterinary diagnosed PPID population compared to studies including solely referral hospital or research herd populations. Furthermore, the sample size is comparatively large compared to previous studies investigating treatment and management of PPID.

In questionnaire-based research, even a small change in question order can have an impact on the answers given by respondents (Lasorsa, 2003; Huang and Cornell, 2016; Lee et al., 2016). This is because each question is not considered in isolation by the participant but in context with those around it (Bowman and Schuldt, 2014; Dillman et al., 2014). Questions measuring subjective concepts such as QoL or well-being have been reported to be particularly affected (Garbarski et al., 2015). This is because participants are unlikely to have pre-defined answers for these types of questions and therefore consult the previous information for context (Tourangeau and Rasinski, 1988; Sudman et al., 1996; Tourangeau et al., 2000). This was demonstrated in a study by Bowling and Windsor (2008), where patients rated their QoL status as significantly better if health based questions were asked beforehand. Therefore, to minimise the influence of question order on over-estimation of

QoL ratings, questions relating to QoL were located close to the beginning of the questionnaire before sections regarding management, health care and concurrent disease.

The horse-owner relationship is unique, as horses are neither a pet in the conventional sense or a livestock animal. In recent years the horse has been considered more of a companion animal, with surveys reporting that some owners value their horse for their companionship and consider them to be like a member of the family (Anon, 2007b; Visser et al., 2012). Furthermore, horses are being cared for into their old age with a significant proportion reportedly kept as kept as companions or retired (McGowan et al., 2010a; Ireland et al., 2011a). This increasing duration of ownership means owners are more likely to develop a strong bond with their aged horse (Ireland et al., 2011a; McGowan and Ireland, 2016). The horse-owner relationship, developed over a prolonged period, is likely to influence decisions made around both routine management and treatment of diseases including PPID. It has been reported that owners are interested in maintaining the health and QoL of their horses into their old age (McGowan et al., 2010b). The results presented here may represent the population of owners who are more likely to seek and finance veterinary treatment for their older animal.

The age and gender demographics described here are comparable with the survey of geriatric horses conducted by Ireland et al. (2011a), which reported the median age of the population was 20 years and 55.1% were geldings. However, breed demographics differ from previous surveys of the general equine population, with UK native/native cross breeds making up a much larger proportion than previously reported (Mellor et al., 1999; Hotchkiss et al., 2007a), and ponies comprising a large proportion of the population. Ponies are reportedly more like to show clinical signs such as laminitis and hypertrichosis (McGowan et al., 2010b) and are over-represented with increasing age (Ireland et al., 2011a), therefore the high proportion of pony breeds in this study is not unexpected.

The proportion of the population kept at livery yards (36.4%) was over double that reported in a survey of British horse owners which reported only 16.2% of horses were kept at livery (Hotchkiss et al., 2007a), but was more comparable to the 27.3% reported by Ireland et al. (2011a) in a survey of geriatric horses and a recent UK equine health survey which reported that nearly half of horses (including animals of all ages) were kept in livery (Slater and Taylor, 2018). This might be due to changes in how people keep horses over the last

decade, with fewer people having access to their own facilities, or because owners who keep their horse on a livery yard are more likely to seek veterinary attention due to the influence of others around them. In human medicine, social networks have been reported to influence help-seeking behaviour. When patients consulted their social network, they were encouraged to seek help and peers pointed out changes that the patients themselves had not noticed (Walter et al., 2014). Being housed at a livery yard may also affect management, as owners might be restricted by yard rules around factors such as turnout and have less freedom to make their own decisions regarding their horse's management. This may influence the management practices described here. However, the grazing and stable management practices for PPID horses were not dissimilar to that of equine populations described in other studies (Mellor et al., 2001; Hotchkiss et al., 2007a; Ireland et al., 2011a).

Overall turnout hours reported here were very similar to the time spent at pasture described by Hotchkiss et al. (2007a). The majority of horses had access to grass/grazing (81.6%), a slightly smaller proportion compared to the 90.4% of geriatric horses reported by Ireland et al. (2011a). This may be due to the perceived increased risk of endocrinopathic laminitis associated with PPID (McGowan, 2010) and the proportion of horses in the current study population reported to have a history of laminitis. Although horses with a history of laminitis spent significantly less time at pasture, the majority of horses still spent a large proportion of time turned out throughout the year. This perhaps demonstrates the effectiveness of the restricted grazing management practices used by 77% of participants, allowing horses to be turned out while also restricting their grass intake (Geor and Harris, 2009).

Diet is considered by owners of older horses to be an important contributor to QoL (Ireland et al., 2011c) and the feeding practices of owners change as their horse ages (Brosnahan and Paradis, 2003a; Ireland et al., 2011a). As the majority of questionnaires were completed during winter and early spring months, and owners were requested to provide information regarding their horse's current diet, the feeding practices reported in this study may not reflect dietary management of PPID cases over the whole year. However, feeding practices reported here were similar to those for the geriatric population reported by Ireland et al. (2011a). The vast majority of horses received some form of bucket/concentrate feed and the most frequently reported feeds were chaff/chop and sugar/fibre beet products in both populations. However, only 13.4% horses in this study were fed veteran/senior feeds

compared to 27.7% reported by Ireland et al. (2011a) and 51% of horses aged ≥20 years in an American study (Brosnahan and Paradis, 2003a). The types of forage fed also differed, with geriatric horses being most frequently fed haylage (52.1%) and only 11.5% receiving soaked hay (Ireland et al., 2011a). In the current study horses were most frequently fed dry hay (49.7%) or soaked hay (31.8%) with only 25.5% being fed haylage. This difference is likely to be linked to owner perception of laminitis risk. Endocrinopathic laminitis is a reported comorbidity of PPID, where concurrent insulin dysregulation and/or hyperinsulinaemia are observed in PPID cases (McGowan et al., 2004; Treiber et al., 2006a; Klinkhamer et al., 2011; de Laat et al., 2019a). In the current population, 28.6% of owners reported laminitis as a reason for suspecting PPID and 16.7% reported their horse had a history of previous laminitis. Feeding practices aimed at reducing calorie intake have been shown to improve insulin sensitivity (Morgan et al., 2016) and are recommended to help prevent endocrinopathic laminitis. The fact owners are choosing to feed less nutritious forage perhaps demonstrates owner awareness of the importance of restricting calorie intake to minimise the risk of laminitis. However, veterinary advice seems to have minimal influence on owner decision making regarding diet, with only a quarter of owners reporting that feeding practices were based on advice from their veterinary surgeon. The majority of owners (67.2%) based decisions regarding diet on their own personal experience. This could be a result of the high amount of value owners place on their own experience and understanding of their individual horse (Scantlebury et al., 2014) meaning owners believe they are best placed to make day to day management decisions.

In a survey of owner-reported BCS in the general equine population, the majority of horses were reported to have a BCS of 4/6 on a 1-6 scale (Robin et al., 2013), whereas the majority of horses in this study had a mean BCS of 2/5 using the same scoring system but a 0-5 scale. This suggests that horses with PPID are likely to have a lower owner-reported BCS compared to the general equine population. This is despite the fact a significant proportion of the population comprised Welsh and other UK native breeds, which are reported to have significantly increased odds of obesity (Robin et al., 2013) and contrary to a previous cross-sectional study which described increasing risk of obesity with increasing horse age (Thatcher et al., 2012). However, the majority of horses aged ≥15 years were reported by their owners to be in good body condition, although BCS decreased significantly with increasing horse age (Ireland et al, 2011a). The lower BCS reported here might be due to several factors. Owners have been reported to underestimate BCS in horses (Wyse et al.,

2008; Ireland et al., 2012a) and clinical signs such as muscle wastage and changes in fat distribution often observed in PPID cases are likely to influence owners' perception of body condition. Determining BCS is likely to be more difficult and less accurate when also accounting for these clinical signs of PPID. To try and combat this, detailed descriptions were provided for each BCS (Carroll and Huntington, 1988) and owners were asked to provide three individual scores, one for each region of the body, which were used to calculate overall mean BCS. Free access to grass has been reported as a risk factor for obesity (Thatcher et al., 2012) and the majority of animals in this study had their access to grass restricted for a least some period during the year. Despite the majority of horses reportedly being in 'average' rather than 'good' body condition, most owners (70.6%) designed their horse's diet to maintain their current weight, suggesting owners were happy with their horse's current weight.

Geriatric horses have been reported to still have a useful working life, with as many as 74% still in ridden work (Ireland et al., 2011a). Similar to several other studies (Mellor et al., 2001; Brosnahan and Paradis, 2003a; McGowan et al., 2010a; Ireland et al., 2011a) the main use of horses reported here was low intensity pleasure riding/hacking. However, the proportion of horses reported to be 'in work' was much lower, with just over half receiving regular exercise. In the general equine population, only 18% were described as being a companion or retired (Mellor et al., 2001). However, this difference is not unexpected, since increasing age has been associated with lower intensity exercise and retirement in various studies (Brosnahan and Paradis, 2003a; McGowan et al., 2010a; Ireland et al., 2011a) and the same association was reported in the current study. This perhaps highlights the continuing shift towards the role of the horse a companion animal and the willingness of owners to care for their horses beyond their 'useful' working life.

Horses had received a median of two routine veterinary visits in the previous 12 months, considerably more the median of 0.5 per year received by the general population (Mellor et al., 2001). Ireland et al (2011b) reported that 68.7% of geriatric horses received a routine veterinary visit within the preceding year and that increasing age and retirement were associated with a reduced likelihood of routine veterinary visits. The results presented here suggest that horses with PPID receive more routine veterinary care than the general geriatric equine population. Clinical examination is required to renew prescriptions and twice yearly ACTH monitoring is currently recommended to assess disease progression and

response to treatment (Durham et al., 2014). As the majority of horses had received pergolide treatment, this recommended monitoring is likely to be one of the reasons for the increased veterinary care reported here. It is likely that veterinary registered horses are over-represented in the study population, therefore it is possible that the results presented here over-estimate the level of veterinary attention provided for the entire population of horses with PPID.

The majority of horses were currently vaccinated against tetanus (75.9%) and equine influenza (67.1%), which although reduced compared to the general population (Hotchkiss et al., 2007b) is broadly comparable to the geriatric population (Ireland et al., 2011b). The likelihood of regular vaccination is reported to decrease with age (Mellor et al., 2001; Ireland et al., 2011b). This could pose a significant health risk as both geriatric horses (Muirhead et al., 2008) and PPID cases (Adams et al., 2014) have been shown to have a reduced immune response to vaccination. Immunosuppression has also been documented as a consequence of PPID (Schott et al., 2001; McFarlane, 2014). This is thought to be a result of high concentrations α-melanocyte stimulating hormone (α-MSH) and insulin. The mechanisms are poorly understood, however, neutrophil activity in horses with PPID has been reported to be reduced compared to healthy adult and aged controls (McFarlane et al., 2015). In light of the increased number of UK equine influenza outbreaks reported during 2019 (Animal Health Trust, 2019) increased emphasis should be put on the importance of vaccinating horses with PPID.

Preventive health care undertaken was comparable with previous studies (Brosnahan and Paradis, 2003a; Ireland et al., 2011b): the majority of owners followed a worming regimen and provided regular dental care. The worming practices described here demonstrate the increasing utilisation of targeted selective worming programmes, with half of owners reportedly worming based on the results of FWECs. This is a marked increase from the 10.9% and 1.8% described in previous studies (Hotchkiss et al., 2007b; Ireland et al., 2011b). A relatively high proportion of horses in this study were reported to receive six monthly dental checks, which is in keeping with studies that have reported an association between the prevalence of dental disorders and increasing age (Ireland et al., 2011b; Ireland et al., 2012b). The importance of regular dental care in horses with PPID has been highlighted (McFarlane, 2011), however any association between dental problems and PPID is not well documented. The sampling method and questionnaire design employed in this

study could mean that horses from large establishments, such as riding schools, are possibly under-represented. This may bias the estimates of frequencies of preventive health care.

A recent systematic review collated data on the most prevalent veterinary-diagnosed clinical signs of PPID, and reported an overall prevalence of hypertrichosis (69.9%; based on data from 14 studies), laminitis (48.9%; based on data from 13 studies) and muscle wastage (48.9%; based on data from four studies) (Ireland and McGowan, 2018). Compared to the current study, the higher prevalence of these clinical signs is most likely because many of the previous studies were case series with study populations often including horses with advanced disease (Ireland and McGowan, 2018). It is also possible that clinical signs in this study were under-reported or unrecognised by owners. This has previously been reported for factors such as coat abnormalities (Ireland et al., 2012a). One Australian survey described owner-reported clinical signs in 69 PPID cases aged ≥15 years: hypertrichosis was reported in 41%, lethargy in 14% and laminitis in 13% of horses (McGowan et al., 2013a). These also differed from the owner-reported reasons for suspecting PPID in the current study, where hypertrichosis was less prevalent and the prevalence of lethargy and laminitis was higher. This could be due to the younger median age of the study population presented here. In this study, horses reported to have an overgrown coat prior to diagnosis were significantly older, while horses with laminitis or lethargy/poor performance were significantly younger than those not displaying these signs. Younger horses are more likely to be in work, therefore owners may be more likely to notice signs of lethargy/poor performance and laminitis is more likely to occur in horses with concurrent EMS (McGowan, 2010c) or hyperinsulinaemia (Karikoski et al., 2016). Additionally, PPID has been diagnosed with increasing frequency in recent years (Rohrbach, et al., 2012) due to increased awareness of the disease. This is likely to lead to a greater proportion of cases being diagnosed in the earlier stages of disease before overt generalised hypertrichosis develops. Since owners were asked to report reasons for suspecting PPID at the time of diagnosis, which was up to five years prior to the questionnaire, some of the differences in clinical signs reported may also be due to recall bias. Owners are more likely to recall significant events (Casey et al., 1967; Erk et al., 2003) which have a high impact and a laminitis episode or lethargy is likely to have a much greater impact on aspects such as daily routine than hypertrichosis.

Pergolide is the treatment of choice for PPID and has been shown by various studies to be effective at improving clinical signs and ACTH concentration in horses with the disease (Chapter 4). It is therefore unsurprising that the majority of the population described here were reported to currently receive pergolide treatment. However, this is in contrast to treatment compliance for chronic conditions in humans which has been reported to be as low as 50% (Miller, 1997). Owners following veterinary advice may have been more likely to respond to the questionnaire, therefore the frequency of pergolide treatment reported in this study might be over-estimated. One of the main reasons for non-compliance in human patients was because medications were considered to be ineffective (Miller, 1997). This is the first study to assess owner perception of treatment efficacy in a relatively large sample of PPID cases. Pergolide treatment was perceived by owners to be highly effective, with a median rating of 8/10. This is in keeping with a previous small retrospective cohort study, which reported that 90% of owners were satisfied with pergolide treatment (Pongratz et al., 2010). Similarly, ten years after commencing treatment, 96% of owners agreed or strongly agreed that pergolide improved their horse's QoL (Schott et al., 2020b). This seems to demonstrate that owners are observing the efficacy described in clinical research and perhaps contributes to the high compliance reported in this study population. Nearly all owners who reported stopping pergolide treatment did so because of observed side effects. Just over half of owners reported observing side effects during the first two weeks of treatment, which is a higher portion than previously described (Anon, 2011a). This could be a consequence of owners perceiving unrelated issues or events as side effects of treatment or because owners are perhaps more likely to notice side effects such as lethargy or changes in behaviour, compared to findings from veterinary examination.

Assessing QoL is a useful tool to help veterinary surgeons make treatment recommendations and monitor longitudinal changes as well as providing an opportunity to assess owner perceptions of disease (Yeates & Main, 2009). Quality of life is considered one of the most important factors affecting owner decision making regarding disease treatment in older horses (Ireland et al., 2011c). The ability to assess individual QoL and factors affecting it is important when managing a chronic disease such as PPID. The importance of individualised QoL assessment has been recognised in small animal medicine (Budke et al., 2008) with several owner assessment tools already developed for dogs and cats (Wiseman-Orr et al., 2004; Tzannes et al., 2008; Freeman et al., 2012; Lavan, 2013; Tatlock et al., 2017). It has been reported that owners' perceptions of QoL and factors influencing it could be affected

by anthropomorphism or anthropocentrism (Bradshaw and Casey, 2007). However, compared to veterinary surgeons, owners have more experience of the individual animal and its normal daily activities, making them better positioned to assess QoL (McMillan, 2003; Wojciechowska and Hewson, 2005). The use of owner ratings in animal welfare research has been reported to be a legitimate and useful tool for assessing QoL (Meagher, 2009). Currently there is no validated way of measuring QoL in horses. Most currently available equine welfare assessment tools (reviewed by Hockenhull and Whay, 2014) focus on assessment of health indicators and clinical parameters, with none specifically designed for completion by horse owners. Therefore, no specific welfare assessment tool was deemed suitable for integration into an owner-completed questionnaire to gather information on owner perceived QoL. Instead, currently available assessment tools informed the QoL domains evaluated in the current study. Questions around QoL were developed from VAS utilised in human medicine (Katsura et al., 2003) and small animal assessment tools (Belshaw et al., 2015) to encompass a range of QoL domains (WHO, 2020). These included, activities of daily living, which have previously been considered to influence QoL in older horses (Ireland et al., 2011c), and the five freedoms (Mellor, 2016).

Horses in this study were considered by their owners to have a very good overall QoL and to be experiencing minimal discomfort. This is similar to the good-excellent QoL rating reported for the general geriatric population (Ireland et al., 2011c). The high QoL rating given, despite the presence of a chronic condition, may be because PPID is perceived to be manageable, similar to diabetes in humans which may have minimal impact QoL if managed correctly (Hänninen et al., 2001). It has been suggested that owners may not be as adept at recognising pain in older horses because they misinterpret clinical signs as being signs of aging (McGowan et al., 2010b) and pain is an important influencer on QoL rating (Ireland et al., 2011c). Additionally, owners of horses with PPID recognise aspects of QoL that are good despite the condition. This has been described in human medicine where patients who have significant health and functional problems do not necessarily have QoL scores that seem to correspond with their health (Carr and Higginson, 2001) because they are able to adapt to overcome the illness (Hyde et al., 2003).

Quality of life rating improved significantly from the time of diagnosis and treatment with pergolide was associated with a higher QoL rating. Although this might infer that pergolide treatment improved QoL ratings, owners were asked to retrospectively rate their horses QoL

at time of diagnosis, which may have introduced a degree of recall bias. Additionally, owners may have expected to see a difference after commencing treatment and therefore the difference in QoL rating may be over-estimated. Several management factors were associated with QoL rating. An average or good BCS was associated with a higher QoL rating, compared to animals reported to be underweight. Weight or BCS is considered and important health issue (Buckley et al., 2004), weight loss is a concern among owners of geriatric horses (McGowan et al., 2010b) and obesity is associated with metabolic health issues (Treiber et al., 2006b). Therefore, the association between BCS and QoL reported here is not surprising. Restricted grazing, particularly non-grass turnout, was negatively associated with QoL rating. For all methods of restricting grazing evaluated in the current study, the proportion of horses considered to have a good QoL (owner rating of overall QoL ≥8/10) was smaller than that for horses with no restrictions to their access to grazing, with the greatest negative effect on QoL for use of a bare/dirt paddock, whereas use of a track system was not significantly different from no restriction. Further investigation is required to indicate if this effect on QoL varies dependent on whether restricted grazing practices are used for all or part of the year. Field turnout and good grazing were reported by owners as factors which positively influenced QoL of geriatric horses (Ireland et al., 2011c). Horses with access to grazing may be perceived by owners to have the freedom to express normal behaviours such as foraging and socialising resulting in a higher QoL rating. Stabling for extended periods of time has been associated with stereotypical and unwanted behaviours (McGreevey et al., 1995a,b). Also, horses with restricted access to grazing are more likely to have had or currently have laminitis, which was significantly association with a reduced QoL. Painful and recurrent conditions, such as laminitis, have previously been reported to influence owner perception of QoL (Ireland et al., 2011c). Several other clinical signs were also associated with a lower QoL rating including a curly/over-grown coat, muscle wastage, lethargy/poor performance and patchy/excessive sweating. It is doubtful that these clinical signs cause pain therefore, their association with QoL is likely to be due to their impact on daily management and owner perception of QoL.

Conclusion

Overall horses with PPID were perceived to have a very good QoL and pergolide treatment appeared to have a positive influence on QoL rating. The majority of horses received veterinary recommended treatment as well as a high level of routine and preventive health

care. Feeding and management practices undertaken was similar to those previously reported for geriatric horses, with the exception of carefully controlled access to grazing. Evaluation of QoL may be beneficial as a component of routine prescription checks or when considering changes in pergolide dose, but there remains the need for a practical tool that allows both owners and veterinary surgeons to easily assess QoL in horses. In the meantime, the impact of PPID on daily routine should be considered alongside clinical factors to maximise QoL in horses diagnosed with PPID.

Appendices for Chapter 5

Appendix 1 – Invitation letter sent to eligible owners registered with the University of Liverpool Equine practice

Dear [client's name],

Further to our initial phone conversation we could like to give you details of a research study being carried out by the University of Liverpool. We are conducting a survey of owners of horses and ponies diagnosed with pituitary *pars intermedia* dysfunction (PPID; also known as Cushing's syndrome).

In our previous research, consultation with vets and owners with experience of PPID has identified important research questions, which you as carers want answered. One of top ten most important research priorities was to investigate the most effective management and treatment strategies for horses and ponies with PPID. We are writing to invite you to participate in a research study we hope will go some way to addressing this important area of research.

The purpose of this study is to improve our knowledge and understanding of current management and treatment approaches undertaken and to develop healthcare plans to improve the quality of life of horses and ponies with PPID. However, this research could not be done without your help. The answers you provide about your horse or pony can help make a real difference to the health of horses and ponies with PPID.

It is important for you to understand why the research is being done and what it will involve. Please take time to read the enclosed study information sheet carefully and feel free to ask us if you would like more information or if there is anything that you do not understand (using the contact details provided below).

The initial survey will be conducted by telephone and consists of questions about your horse's routine, feed, general health and, where applicable, treatment for PPID. We will also ask you to complete three short follow-up questionnaires to document any changes over time. Your privacy is, of course, of the utmost importance to us and if you decide to take part, all responses are completely confidential and will only be used for the purposes of this survey.

We hope you will be able to participate, however your involvement is entirely voluntary. If you no longer wish to take part please email rebecca.tatum@liverpool.ac.uk or telephone Becky on 01638 751000 extension 1241, we will not contact you further and the contact details you provided will be removed from our dataset. If we do not hear from you the University of Liverpool researcher, Becky Tatum, will contact you by telephone at your specified convenient time.

Thank you for your support so far.

Yours sincerely,

Becky Tatum, PhD Student, University of Liverpool

Dr Joanne Ireland, PhD Supervisor, University of Liverpool

Professor Catherine McGowan, PhD supervisor, University of Liverpool

Appendix 2 - Information sheet sent alongside invitation letter to eligible owners registered with the University of Liverpool Equine practice

Information Sheet

Optimising Care for Cushing's (PPID) Horses and Ponies

You are being invited to participate in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and feel free to ask us if you would like more information or if there is anything that you do not understand. Please also feel free to discuss this with your friends, relatives or veterinary surgeon if you wish. We would like to stress that are free to opt out of participation and should only agree to take part if you want to.

What is the purpose of the study?

The purpose of this study is to investigate which management and treatment factors are most likely to improve quality of life for horses and ponies with pituitary pars intermedia dysfunction (PPID; also known as Equine Cushing's Syndrome). Quality of life is a way of measuring your horse/pony's overall health, wellbeing and happiness.

Why have I been chosen to take part?

You have been chosen to take part in this study because your horse/pony has been diagnosed with PPID (based on a laboratory test result where the hormone ACTH was above the normal reference range for the time of year the sample was taken). If this information is no longer correct, please inform us of any change in circumstances using the contact details below. All owners registered with the University of Liverpool Leahurst Equine Practice whose horse/pony has been diagnosed with PPID in the past three years are being invited to participate in this study. All horses/ponies diagnosed with PPID are eligible to be included in this study, whether or not they are currently receiving any medical treatment for the condition.

About the study

If you agree to take part in the study, we will ask you to take part in a telephone questionnaire, which will take around 20 minutes to complete. You will be asked questions about your horse/pony's routine, daily care, exercise, health, any medication or other treatments they receive and how you perceive their quality of life. This will be followed by three shorter follow-up questionnaires at approximately three month intervals to document any changes over time. Your responsibility as a participant of this study will be to answer all questions honestly and with your own opinion. We will also use your horse/pony's clinical records from the Leahurst Equine Practice in order to help us understand the associations between PPID laboratory test results (measuring ACTH) and health and quality of life of horses/ponies with PPID.

Participating in this study will not interfere with your horse/pony's management, routine preventive healthcare, treatment for PPID or treatment of any other conditions. However, if you chose to make any changes to your horse/pony's management throughout the study, such as feeding, exercise regime, field turnout or stabling and any healthcare you provide such as worming or vaccinations, we would ask you to record these changes and let us know, either directly using the contact details provided, or during a follow-up telephone questionnaire.

The data we collect will be anonymised with no information that could identify you or your horse. It will be kept confidential and stored securely on University password protected computers, it will be used for this specific study only and only the study research team at the University of Liverpool will have access to the data. You or your horse/pony will not be identifiable from the results.

Why is this study important?

Pituitary pars intermedia dysfunction is the most common hormonal disorder of older horses/ponies. Previously we have engaged with vets and owners (with experience of PPID) to prioritise areas of research they consider to be important. Establishing which management (including aspects such as dietary and turnout) and treatment strategies are most effective were areas ranked in the top 10 research priorities. The information provided in this study will help us to better understand current management and treatment approaches undertaken for horses/ponies with PPID. The aim is to have more information about management and treatment options for veterinary surgeons and owners in order to improve the quality of life of horses and ponies with PPID.

Are there any risks to myself or my horse/pony?

As we are only using telephone questionnaires to collect data we do not anticipate any disadvantages or risks associated with your or your horse/pony's participation.

What happens if I want to stop taking part?

We would like to stress that your participation is voluntary. As a participant if you wish to end your participation at any point during the phone call or duration of the study you are free to do so. If you decide to withdraw your responses during or at the end of the interview, and if you request it, we will destroy any data regarding yourself or your horse from the study.

Contact details

If you have any questions or for further information please contact:

Becky Tatum

Email: rebecca.tatum@liverpool.ac.uk

Telephone: 01638 751000 Ext. 1241

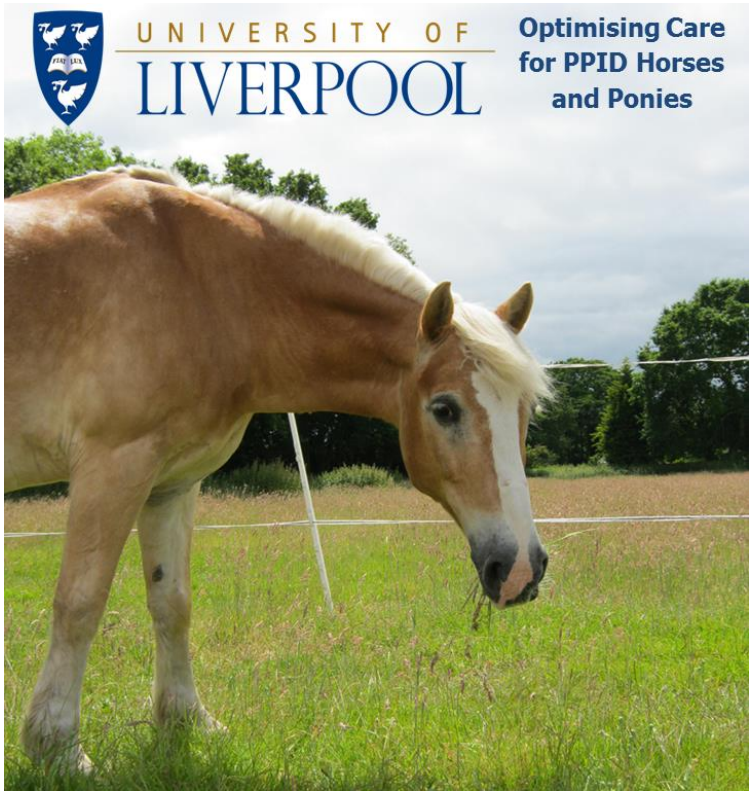
If you are unhappy, or if there is a problem, please feel free to let us know by contacting Becky Tatum or Jo Ireland and we will try to help. If you remain unhappy or have a complaint which you feel you cannot come to us with then you should contact the Research Governance Officer on 0151 794 8290 (ethics@liv.ac.uk). When contacting the Research Governance Officer, please provide details of the name or description of the study (so that it can be identified), the researcher(s) involved, and the details of the complaint you wish to make.

Thank you for taking the time to read this.

Becky Tatum

The contact details of lead Researcher (Principal Investigator) are: [Contact: Dr Joanne Ireland, Leahurst Equine Practice, Leahurst Campus, University of Liverpool, Neston, Wirral, CH64 7TE, email: joanne.ireland@liverpool.ac.uk]. If there are any problems, please let us know and we will try to help.

Appendix 3 - The image and text used to promote the survey on the UoLEP and Talk About Laminitis Facebook pages



Can you help improve the care of horses and ponies with PPID (Equine Cushing's Syndrome)

If your horse or pony has been diagnoses with PPID within the last 4 years please help us by completing our questionnaire by following this link: https://kwiksurveys.com/s/xRjV8Vdm

Pituitary Pars Intermedia Dysfunction (PPID also known as Cushing's) is the most common hormonal disorder in older horses and ponies in the UK. Here at the **University of Liverpool** we are conducting an important new research study called; **Optimising Care for PPID Horses and Ponies**.

The purpose of this study is to **improve our knowledge and understanding** of current management and treatment approaches undertaken by owners and carers. This will help to **develop healthcare plans to improve the quality of life** of horses and ponies with PPID.

For more information and to take part follow this link: https://kwiksurveys.com/s/xRjV8Vdm

Picture to accompany post:

Appendix 4 - The questionnaire used to gather information regarding owner management practices and QoL in horses with PPID.

Optimising Care For PPID Horses and Ponies

Baseline Questionnaire

The University of Liverpool is conducting an important new research study called; Optimising Care For PPID Horses And Ponies.

If your horse or pony has been diagnosed with PPID (also known as Equine Cushing's Syndrome) within the last 4 years please help us with this important research by completing the following questionnaire regarding your horse's routine, management, health and healthcare.

The purpose of this study is to improve our knowledge and understanding of current management and treatment approaches undertaken by owners and carers. This will help to develop healthcare plans to improve the quality of life of horses and ponies with PPID.

About you

Please confirm your contact details:

Name

Address

Address

Town

County

Post code

Email address

Phone number(s)

1.General information about your horse

1 What is your horse's name?

2 How long have you owned/cared for your horse? (If unsure please give an approximate number of years/months)

3 How old is your horse? (If unsure please give an approximate age in years)

4 What breed is your horse?

5 What height is your horse in hands? (if unsure please give an approximate height)

6 What gender is your horse?

A	Mare	B	Gelding	C	Stallion

7 Please indicate which best describes where your horse is usually kept

A	Own premises (at home)	B	Private yard	C	Livery yard
D	Rented premises/pasture	E	Riding school	F	Competition yard
G	Farm (livestock or arable)	H	Stud farm		
	Other (Please Specify)				

2. About your horse's diagnosis of PPID

8 When was your horse diagnosed with PPID? (mm/yy or number of years ago)

9 Was your horse diagnosed with PPID using a blood test?

A	Yes	B	No	C	Don't know

10 If your horse was diagnosed using a blood test, what type of blood test was used (select all that apply)?

A	Resting ACTH (Adrenocorticotropic hormone) level	B	TRH (Thyrotropin releasing hormone) stimulation test
C	Overnight dexamethasone suppression test (ODST)	D	Don't know
	Other (Please Specify)		

11 If you remember the test result(s), please indicate them below.

12 What was your reason for first suspecting you horse/pony had PPID (select all that apply)?

A	Age	B	Vet suspected/suggested
C	Active laminitis	D	Previous history of laminitis/hoof changes resulting from previous laminitis episodes
E	Abnormal fat distribution (e.g. fat pads around the neck or hind quarters)	F	Pot belly
G	Wasted/loss of muscle over topline	H	Fat pads around the eyes/puffy lower eye lids
I	Excessive/patchy sweating	J	Increased drinking and urination
K	Lethargy/poor performance	L	Recurrent infections (e.g. foot abscesses, sinusitis)
M	Abnormal coat shedding	N	Curly coat
O	Overgrown coat	P	Changes to coat colour/texture
	Other (Please Specify)		

3.Your horse's treatment for PPID

13 Has your horse ever received pergolide (Prascend) treatment at any time?

A	Yes
B	No

The rest of this page is about treatment with pergolide (Prascend), if your horse has never received pergolide (Prascend) at any time please go to section 4 on the next page.

14 Does your horse currently receive pergolide (Prascend) treatment?

A	Yes
B	No

If your horse does not currently receive pergolide (Prascend) please go to Q18 below.

15 Please indicate the dose of pergolide (Prascend) your horse currently receives (number of tablets and how many times per day)

Number of tablets

Number of times per day

16 Has the above dose of pergolide (Prascend) changed in the last 12 months?

A	Yes, increased	B	Yes, decreased	C	No not changed
	Other (Please Specify)				

17 Did you consult your vet before making changes to the dose of pergolide (Prascend) your horse recieves?

A	Yes	B	No	C	Not applicable dose has not changed in the last 12 months
	Other (Please Specify)				

18 If your horse does not currently receive pergolide (Prascend) when has he/she done so in the past? (If your horse currently receives pergolide (Prascend) please go to Q20 below)

A	When initially diagnosed	B	At various times in the past
C	Dependent on cost	D	At specific times of year (seasonally)
E	Not sure/can't remember		
	Other (Please Specify)		

19 If your horse does not currently receive pergolide (Prascend) please indicate any reasons for your decision (select all that apply).

A	Veterinary advice	B	Do not feel I need to medicate currently
C	Advice from non-veterinary personnel (e.g. friends, other horse owners)	D	Advice from online forums/social media
E	Not palatable (couldn't get my horse to eat the tablets)	F	Side effects observed
G	Not practical	H	No obvious benefit/improvement observed
I	Cost	J	Previous experience of PPID
K	Ability to compete		
	Other (Please Specify)		

20 During the first 2 weeks of treatment did you notice any adverse effects (side effects) of treating your horse with pergolide (Prascend) (select all that apply)?

A	None	B	Loss of appetite	C	Lethargy/poor performance
D	Erratic/unpredictable behaviour	E	Diarrhoea/loose droppings	F	Colic
G	Not applicable my horse has never been treated with pergolide				
	Other (Please Specify)				

21 After the first 2 weeks of treatment did you notice any adverse effects (side effects) of treating your horse with pergolide (Prascend) (select all that apply)?

A	None	B	Loss of appetite	C	Lethargy/poor performance
D	Erratic/unpredictable behaviour	E	Diarrhoea/loose droppings	F	Colic
G	Not applicable my horse has never been treated with pergolide				
	Other (Please Specify)				

22 Did these adverse effects (side effects) resolve?

A	Not applicable no side effects observed	B	Yes, quickly	C	Yes, slowly
D	Improved but not gone completely	E	No		
	Other (Please Specify)				

23 Overall how effective do you feel pergolide (Prascend), treatment is/was for your horse (use the slider below to specify)?

0 1 2 3 4 5 6 7 8 9 10

Not at all effective Extremely effective

24 Please give any reason(s) for your answer to Q23.

4. Your horse's treatment for PPID - Part 2

25 Please indicate if your horse currently receives any other veterinary prescribed treatments for PPID?

A	None	B	Cyproheptadine (Periactin)	C	Trilostane (Vetoryl)
	Other (Please Specify)				

26 Does your horse currently receive any alternative treatments for PPID? (e.g. non-prescription treatments)

A	None	B	Agnus castus/chaste berry	C	Homeopathy
D	Herbal supplements (e.g. Cush aid, Cush-X)	E	Magnesium	F	Antioxidants (e.g. vitamins E and C)
	Other (Please Specify)				

27 Has your horse previously received any alternative treatments for PPID? (e.g. non-prescription treatments)

A	None	B	Agnus castus/chaste berry	C	Homeopathy
D	Herbal supplements (e.g. Cush aid, Cush-X)	E	Magnesium	F	Antioxidants (e.g. vitamins E and C)
	Other (Please Specify)				

28 If your horse did/does receive any alternative treatments, how effective do you feel they are/were for your horse (use the slider below to specify)?

0 1 2 3 4 5 6 7 8 9 10

Not at all effective Extremely effective

29 Please give a reason(s) for your answer to Q28.

30 What factor(s) currently influence your decisions regarding your horse's treatment options for PPID (select all that apply)?

A	Advice from your usual vet	B	Advice from a vet at an equine hospital/other vet practice
C	Advice from non-veterinary personnel (e.g. friends, other horse owners, yard staff)	D	Previous experience of PPID
E	Cost of treatment	F	Age of your horse
G	Prognosis	H	Horse's quality of life
I	Ability of horse to compete	J	ACTH test results
K	Changes in symptoms	L	Online forums/social media
	Other (Please Specify)		

5. Your horse's quality of life

Quality of life refers to your horse's standard of health, comfort, and happiness.

31 How would you have rated your horse's quality of life at the time they were diagnosed with PPID?

0 1 2 3 4 5 6 7 8 9 10

Could not have been worse — Could not have been better

The following questions refer to your horse's current quality of life.

32 How would you rate your horse's current demeanour (their way of looking, behaving and their attitude)?

0 1 2 3 4 5 6 7 8 9 10

Could not be worse — Could not be better

33 How would you rate your horse's current appetite?

0 1 2 3 4 5 6 7 8 9 10

My horse has a poor appetite and does not eat enough — My horse has a good appetite and always wants to eat

34 How would you rate the level of discomfort your horse is currently experiencing?

0 1 2 3 4 5 6 7 8 9 10

No discomfort — Serious discomfort e.g. acute laminitis

35 How would you rate your horse's current overall quality of life?

0 1 2 3 4 5 6 7 8 9 10

Could not be worse — Could not be better

36 Do you feel that PPID affects your horse's normal daily activities?

0 1 2 3 4 5 6 7 8 9 10

Not at all — An extreme amount

37 What do you consider to be the most important factor(s) that may have influenced your horse's quality of life since being diagnosed with PPID?

6. About your horse's health regime

38 How do you decide on a worming regime for your horse?

A	I worm based on faecal egg counts	B	I worm when the vet advises me to	C	I worm when the yard manager/owner tells me to or when everyone else at the yard worms
D	I always worm at the same time(s) each year	E	I did my own research and worm based on that	F	I do not have a worming regime
	Other (Please Specify)				

39 If you use faecal worm egg counts, when did you last have one done? (dd/mm/yy)

40 If you can remember the result of your horses latest faecal worm egg count, please give details below

41 When was your horse last wormed? (dd/mm/yy)

42 Which wormer did you use?

A	Equest	B	Equest pramox	C	Equitape
D	Eqvalan	E	Noromectin	F	Panacur
G	Strongid-P	H	Strongid-P double dose	I	Other

43 How often do you have your horse's teeth checked?

A	Every 6 months or more regularly	B	Once yearly	C	Occasionally (e.g. every 2 years)
D	Only when you suspect a problem	E	Not checked		

44 Who usually checks your horses teeth?

A	Vet	B	Vet who has a further dentistry qualification	C	Dental technician
	Other (Please Specify)				

45

Does your horse have any dental problems?

A	Yes	B	No

46 If yes, please give details of your horse's dental problem(s)

47 Is your horse currently vaccinated against any of the following (select all that apply)?

A	Influenza (Flu)	B	Tetanus	C	Strangles
D	Equine Herpes Virus (EHV)	E	No		

48 Do you undertake any other management strategies to help keep your horse comfortable and healthy?

7. Veterinary Visits

49 How many routine vet visits/examinations has your horse had in the last 12 months? (e.g. routine vaccination, prescription check)

50 How many vet visits has your horse had for health problems/emergency(s) in the last 12 months?

51 If the vet attended your horse for a health problem in the last 12 months, please indicate which best describes the problem (select all that apply)

A	Lameness (excluding laminitis)	B	Laminitis	C	Infection (e.g. foot abscess, sinusitis)
D	Injury (e.g. cut, kick)	E	Colic	F	Respiratory disease (e.g. asthma, RAO)
G	Other illness (e.g. virus, flu)				
	Other (Please Specify)				

52 Has your horse been diagnosed with equine metabolic syndrome (EMS)?

A	Yes	B	No	C	Don't know

53 Does your horse have any other health conditions?

54 Is your horse currently showing any clinical signs (symptoms) of PPID (select all that apply)?

A	None	B	Active laminitis
C	Hoof changes from previous history of laminitis	D	Abnormal fat distribution (e.g. fat pads around the neck or hind quarters)
E	Pot belly	F	Wasted/loss of muscle over topline
G	Fat pads around the eyes/puffy lower eye lids	H	Excessive/patchy sweating
I	Increased drinking and urination	J	Lethargy/poor performance
K	Recurrent infections (e.g. foot abscesses, sinusitis)	L	Abnormal coat shedding
M	Curly coat	N	Overgrown coat
O	Changes to coat colour/texture		
	Other (Please Specify)		

8. Turnout and outdoor management

Please note that turnout refers to free access to any uncovered space outside the stable including grass paddock, bare/dirt paddock , surfaced area etc.

55 Which best describes how your horse is usually kept during the spring/summer months?

A	Outdoors full time	B	Outdoors during the day/stabled at night	C	Stabled during the day/outdoors at night
D	Entirely stabled/indoors	E	Outdoors with free access to shelter/indoor area		

56 Which best describes how your horse is usually kept during the autumn/winter months?

A	Outdoors full time	B	Outdoors during the day/stabled at night	C	Stabled during the day/outdoors at night
D	Entirely stabled/indoors	E	Outdoors with free access to shelter/indoor area		

57 On average how many hours per day does your horse spend turned out throughout the year? (If your horse is kept entirely stabled/indoors please go to section 9 on the next page)

- Spring (number of hours)
- Summer (number of hours)
- Autumn (number of hours)
- Winter (number of hours)

58 Do you use any methods to restrict grass intake (select all that apply)?

- A Grazing muzzle (dependent on time of year)
- B Grazing muzzle (all year round)
- C Strip grazing (dependent on time of year)
- D Strip grazing (all year round)
- E Time-restricted grazing (dependent on time of year)
- F Time-restricted grazing (all year round)
- G Track system grazing (dependent on time of year)
- H Track system grazing (all year round)
- I Bare/dirt paddock (dependent on time of year)
- J Bare/dirt paddock (all year round)
- K No
- Other (Please Specify)

59 Which type of turnout does your horse currently have access to?

- A Grass paddock
- B Bare/dirt paddock
- C Soft surface area
- D Hard surface area
- Other (Please Specify)

60 What size is your horse's current turnout area? (Please choose an approximate size category)

- A Small paddock (less than 1 acre)
- B Medium paddock (1-3 acres)
- C Large paddock (4-6 acres)
- D Very large paddock (over 6 acres)

61 Is your horse currently turned out individually or with other horses?

- A Individually
- B With one other horse
- C With between 2-5 other horses
- D With more than 5 other horses

9. Stabling and indoor management

If your horse is kept entirely outdoors/turned out please go to section 10 on the next page.

62 What is the main reason for stabling your horse for all or part of the time?

A	Weather conditions	B	To prevent injury	C	Reduce grass intake
D	Personal preference	E	Preserve/rest grazing	F	Yard rules
	Other (Please Specify)				

63 How is your horse usually stabled?

A	Loose box/stable open fronted	B	Loose box/stable in an American-type barn	C	Loose box/stable in a converted barn/outbuilding
D	Communal indoor space	E	Field shelter (used as a stable/not freely accessible)		
	Other (Please Specify)				

64 On average how many hours per day does your horse spend stabled throughout the year?

Spring (number of hours)
Summer (number of hours)
Autumn (number of hours)
Winter (number of hours)

65 Which bedding(s) do you usually use?

A	Straw	B	Wood shavings	C	Paper
D	Wood pellets	E	Hemp/flax	F	Rubber matting
	Other (Please Specify)				

10. About your horse's feeding

66 Which type(s) of hard feed do you regularly give your horse (select all that apply)?

A	Chaff/chop	B	Coarse mixes	C	Over sixteen/veteran mix/cubes
D	Fibre/grass nuts/cubes	E	Cereals (oats, barley, maize)	F	Alfalfa
G	Sugar beet/fibre beet	H	Bran	I	Balancer
J	PPID/laminitis specific feeds (e.g. Antilam, L mix, CushCare)	K	Conditioning cubes/mix	L	Mash feeds
M	None				
	Other (Please Specify)				

67 How often does your horse currently receive hard feed/bucket feed?

A	Never	B	Once a day	C	Twice a day
D	Three times a day	E	More than three times a day		

68 Which type(s) of forage (other than grass) does your horse currently receive?

A	Dry hay	B	Soaked hay	C	Steamed hay
D	Haylage	E	Hay replacer (e.g. Hi-Fi, readigrass)	F	Straw
G	None (grass only)				
	Other (Please specify all forages)				

69 How often does your horse currently receive forage?

A	Freely available (ad lib)	B	Once a day	C	Twice a day
D	Three times a day	E	More than three times a day	F	Not applicable - not currently fed supplementary forage
	Other (Please Specify)				

70 How is forage currently offered?

A	Free from the ground	B	Straight from the bale	C	Regular holed haynet
D	Small holed haynet	E	Manger/hay rack	F	Not applicable
	Other (Please Specify)				

71 How do you determine how much forage to feed your horse daily?

A	Number of sections/leafs	B	Estimated amount/weight	C	Weighing scales
D	Ad Lib (as much and as often as desired)	E	Not applicable I do not feed forage		
	Other (Please Specify)				

72 Taking into consideration your answers above, have you designed your horse's diet to;

A	Maintain their current weight	B	Decrease their weight	C	Increase their weight
D	I have not consciously designed their diet				

73 Does your horse currently receive any supplements? (select all that apply)

A	None	B	General/multi vitamin and minerals	C	Calmer
D	Electrolytes	E	Joint supplement	F	Muscle building supplement (e.g. Myoplast)
G	Oil supplement	H	Hoof supplement	I	Herbal supplement
J	Garlic	K	PPID/Cushings supplement	L	Turmeric
	Other (Please Specify)				

74 How did you decide what to feed your horse?

A	Personal experience	B	Vet adivce	C	Nutritionist advice (over the phone or a yard visit)
D	Advice from another equine professional (e.g. your instructor, yard manager)	E	Advice from a friend	F	Online information
G	Magazine articles				
	Other (Please Specify)				

11. About your horse's body condition

To help with ease and accuracy we have divided the horse into three sections; neck, ribs/stomach and hind quarters. Please choose the option which best describes each section of your horse.

75 Which option best describes the condition of your horse's neck?

A	No visible crest	B	Visible crest but fat is distributed evenly	C	Crest is thickened with fat deposited more in the middle
D	Crest is very enlarged/thickened	E	Crest is extremely large and can droop to one side		

76 Which option best describes the condition of your horse's ribs/stomach?

A	Ribs easily visible	B	Ribs just visible	C	Pot belly (carrying weight low with ribs visible)
D	Ribs covered with little fat	E	Ribs well covered	F	Ribs very well covered/buried

77 Which option best describes the condition of your horse's hind quarters?

A	Prominent pelvis and sunken rump	B	Flat rump with little fat	C	Rounded rump evenly covered with fat
D	Rounded rump with uneven fat distribution	E	Very rounded rump with a gutter along to the base of the tail	F	Heart shaped rump with fat pads and very deep gutter to the base of the tail

78 Do you use any of the following methods to monitor your horse's weight?

A	Weight tape	B	Weight measurements/formulas	C	Body condition scoring
D	Weigh bridge	E	None, do it by eye		
	Other (Please Specify)				

79 How often do you monitor your horse's weight?

A	Regularly	B	Occasionally
C	Rarely	D	Never

12. About your horse's exercise

80 What is your horse currently used for (select all that apply)?

A	Hacking/pleasure riding	B	Riding school/trekking	C	Riding/pony club activities
D	Hunting	E	Breeding	F	Companion/pet
G	Showing	H	Showjumping	I	Endurance/TREC
J	Driving	K	Retired	L	Dressage
M	Eventing	N	Polo		
	Other (Please Specify)				

81 Do you currently compete your horse in any of the above activities? (If your horse is not usually exercised please go to question 85 below)

A	Yes	B	No

82 On average, how many days a week do you currently exercise your horse?

83 On average, how long do you currently exercise your horse for on each occasion?

84 What intensity exercise does your horse currently receive?

A	None	B	Low (mostly walking with some trotting)	C	Medium (trotting, cantering, low jumps)
D	High (galloping, jumping, high level dressage)				

85 If your horse is retired or not usually in work/exercise please indicate why (e.g. retired due to injury, brood mare)

13. About your horse's shoeing/trimming

86 How often is your horse shod/trimmed? (e.g. every 6 weeks)

87 How is your horse currently shod?

- A All four feet
- B Front feet only
- C Barefoot/not shod

88 Is your horse currently shod with any remedial shoes?

- A No/not applicable
- B Egg-bar shoes
- C Heart-bar shoes
- D Straight-bar shoes
- E Glue on Shoes
- Other (Please Specify)

89 If yes, what is the reason for the remedial shoes?

90 Who usually shoes/trims your horses feet?

- A Farrier
- B Barefoot trimmer
- C Vet
- D No-one/not trimmed
- E I do this myself
- Other (Please Specify)

91 Has your horse previously been lame or foot-sore immediately after shoeing or trimming?

- A Yes
- B No

92 Please use the space below to provide any further information you think is relevant to the treatment, management or care of your horse with PPID.

Thank you for completing this Questionnaire and contributing towards our research into PPID

Chapter 6

A prospective cohort study of horses diagnosed with pituitary pars intermedia dysfunction in the United Kingdom

A prospective cohort study of horses diagnosed with pituitary pars intermedia dysfunction in the United Kingdom

Summary

The expected disease progression of PPID, both with and without treatment, has been identified as the most important research priority for owners and veterinary surgeons with experience of PPID. However, information regarding disease progression, management and prognosis is currently limited. Owners of horses diagnosed with PPID were identified through University of Liverpool Equine Practice (UoLEP) database and invited to participate. 84.8% of owners (n=67/79) completed a baseline questionnaire, providing data for 72 PPID cases that were enrolled in the prospective cohort. At least one complete follow-up questionnaire was available for 62 cases that contributed a total of 53.4 horse-years at risk (HYAR), with a median duration of follow-up of 330.7 days (IQR 247.1-388.9 days). Throughout the follow-up period, pergolide dose remained the same for 78.2% (n=43/55) of cases. Quality of life rating remained unchanged in 43.5% (n=27/62) of horses, decreased in 21.0% (n=13/62), increased in 8.1% (n=5/62) and fluctuated in 27.4% (n=17/62). The most frequently reported clinical signs reported throughout follow-up were recurrent/persisting infection (19.4% n=12/62), abnormal coat shedding (16.1% n=10/62) and laminitis (12.9% n=8/62). During the follow-up period 17.7% (n=11/62) of horses died representing an overall mortality rate of 20.6 deaths per 100 HYAR (95% CI: 10.3-36.9 per 100 HYAR). Based on the first new episode during the follow-up period, incidence of laminitis was estimated to be 15.7 episodes per 100 HYAR (95% CI 6.8-30.9 per 100 HYAR) and the incidence of infection was estimated to be 24.9 events of infection per 100 HYAR (95% CI 12.8-43.4 per 100 HYAR). The prospective changes described in this cohort provide valuable additional information on the management, treatment and clinical factors observed in horses with PPID as well as the long-term practices undertaken by owners. This will be useful for veterinary surgeons when advising on the long-term management and prognosis of horses with PPID.

Introduction

Diseases that are prevalent in older horses are becoming important in equine practice (Ireland et al., 2012c; McGowan et al., 2013a), since the equine population is living longer

(Welsh et al., 2016). Pituitary pars intermedia dysfunction (PPID) is a progressive endocrine disease caused dopaminergic neurodegeneration that is frequently diagnosed in older horses (aged ≥15 years) (Brosnahan and Paradis, 2003b; McGowan et al., 2013a). It is characterised by a wide variety of clinical signs including hypertrichosis, epaxial muscle wastage and lethargy (McFarlane, 2014; Ireland and McGowan, 2018), and its progressive nature means long-term management is required.

Expected disease progression, both with and without treatment, has been identified as the most important research priority for owners and veterinary surgeons with experience of PPID (Chapter 2). In human medicine, patient and public involvement (PPI) is regularly used to prioritise and direct research for chronic diseases, helping to improve patient care for conditions such as diabetes and heart disease (Crowe, 2009; Cowan, 2013; Finer et al., 2017). This methodology was adapted into an equine veterinary setting to prioritise research questions for the diagnosis, treatment and prognosis of PPID (Chapter 2). Numerus studies have investigated the diagnosis and treatment of PPID (Donaldson et al., 2002; Pongratz et al., 2010; Anon, 2011a; McGowan et al., 2013b; Rendle et al., 2013; Gehlen et al., 2014), however information regarding disease progression, management and prognosis is currently limited to retrospective data (Welsh et al., 2016), small samples sizes (Rohrbach et al., 2012; Schott et al., 2020b), case series (Spelta and Axon, 2012) or with a referral hospital bias (Horn et al., 2019). PPID has been reported to have a considerable negative effect on survival (Welsh et al., 2016), and associated with an increased risk of mortality (Ireland et al., 2011d; Welsh et al., 2016; Pollard et al., 2020). Rates and causes of mortality have been described for the general geriatric population (Ireland et al., 2011d), with an overall mortality rate of 11.1 per 100 horse-years at risk (HYAR) reported, while lameness (24%) and colic (21%) were the most frequent reasons for euthanasia. However, information regarding incidence of concurrent diseases as well as the incidence and causes of mortality in horses diagnosed with PPID is limited.

Poor owner-reported QoL has also been associated with an increased risk of mortality in geriatric horses (Ireland et al., 2011c). However, despite the impact PPID has on equine health, horses with the disease are perceived by their owners to have a good QoL, as indicated by high ratings of overall QoL and general demeanour, together with low ratings for negative impacts on health-related QoL such as discomfort (Chapter 5). One small study reported that the vast majority of owners would choose to treat another horse with PPID,

and used this to infer that owners were satisfied with long-term QoL (Rohrbach et al., 2012). Other studies have followed PPID cases prospectively and reported good clinical improvement (Schott et al., 2014; Horn et al., 2019) however, individual clinical signs were not investigated.

There is a need for further information regarding owner disease management and clinical changes over time in horses with PPID. This could be utilised to help inform and improve management and treatment decisions, which in turn may improve prognosis. This observational, retrospective and prospective cohort study aimed to describe the changes in treatment, management, clinical factors and QoL observed overtime in horses with PPID as well as incidence of disease and mortality.

Materials and methods

Selection of the study population and retrospective data collection

A review of the University of Liverpool Equine Practice (UoLEP) database was undertaken to identify all PPID cases diagnosed between 1st January 2014 and 31st August 2018 (Figure 1). Cases were diagnosed using basal plasma ACTH concentration above the upper limit of seasonally adjusted reference intervals (>29.7 pg/ml November-July or >47 pg/ml August-October) or a positive response to the administration of thyrotropin-releasing hormone (TRH) (>110 pg/ml at 10 minutes). Horses diagnosed prior to 1st January 2014 were excluded to reduce the risk of owner recall bias. All owners of eligible cases were invited to participate in the study, therefore a sample size calculation was not performed. All owners were initially contacted by telephone and asked if they would like to opt in to the cohort study. Owners opting in at this stage were subsequently sent an enrolment letter and participant information sheet (Appendix 1), which contained details about the study and a further option to opt out if they no longer wished to participate. This study was awarded institutional ethical approval on the 18th May 2018, reference number VREC667.

Owners verbally agreeing to participate during the initial telephone call were also asked whether they would consent to their horse's clinical records being used for the purposes of the research study. Where owners provided consent, data collected from clinical records, included signalment, date of PPID diagnosis, method of diagnosis, ACTH concentration,

owner-reported clinical signs, history of laminitis, clinical examination findings and details of any treatment(s) administered, prescribed or recommended.

Cohort study design and prospective data collection

Prior to commencement of the study >100 cases of PPID diagnosed within the previous four years had been identified on the UoLEP database (Figure 1). In order to optimise recruitment and retention, participating owners were contacted by the doctoral student via telephone to complete the baseline questionnaire, at least two weeks after receipt of the enrolment letter to allow ample time to opt out. Design of the baseline questionnaire is described in full in Chapter 5. In brief, the baseline questionnaire gathered data on stabling/turnout routine, feed, exercise, general health, medical treatment, alternative treatments and owner-perceived QoL. Data were collected on previous clinical signs observed as well as any clinical signs which occurred during the follow-up period. Consecutive prospective follow-up questionnaires were completed over an 18-month period. Both the baseline (Chapter 5 Appendix 4) and follow-up questionnaires (Appendix 2) were designed using online data capture software (KwikSurveys). Where owners were not contactable by telephone within five separate attempts, a link to the baseline questionnaire was sent via email and owners were requested to complete the questionnaire online. Owners who were not contactable by telephone and did not respond to the email requesting online completion of the baseline questionnaire were excluded from the prospective phase of the cohort study.

Owners were then contacted by telephone within 3 – 4 months of the initial baseline questionnaire to complete the first follow-up questionnaire, with three subsequent follow-ups conducted at 3 – 4 month intervals. Again, where owners were not contactable by telephone within five separate attempts, a link to the follow-up questionnaire was sent via email. Owners who were not contactable to complete a minimum of one follow-up questionnaire were excluded from the follow-up study. All telephone questionnaires were administered by the doctoral student.

Data were collected on any changes to management, treatment and health since the previous questionnaire. The Likert-style questions used to gather BCS ratings and the visual analogue scale (VAS) QoL questions were replicated from the baseline questionnaire (See Chapter 5 Appendix 4) and the comprehensive clinical signs list was also included to record

clinical signs observed at the time of follow-up questionnaire. Additionally, episodes of disease or injury were recorded. Where applicable, respondents completed additional questions to gain information regarding the disease episode or injury reported and any treatment administered. The horse's survival status was also confirmed, and where death was reported, owners completed a separate mortality questionnaire (Appendix 3).

All owners who opted in to participate in the study gave consent to obtain their horse's veterinary clinical history. Therefore, the data provided by respondents for ACTH levels at time of diagnosis, episodes of disease, injury or death were cross-referenced with clinical notes and dates of veterinary visits during the follow-up period.

Data analysis

All baseline and follow-up questionnaire data were exported into Microsoft Excel spreadsheets, using the KwikSurveys software, and each horse was allocated a unique identification number. Relevant clinical record data, including ACTH assay results, number of diagnostic and monitoring tests/visits, clinical signs and concurrent conditions, for all horses that entered the study were manually extracted from each individual horse's UoLEP clinical history into a specifically designed Microsoft Excel spreadsheet. Results of all questionnaires and relevant clinical history for each individual horse were then merged into a single spreadsheet for comparison and analysis. Statistical analyses were performed using commercial statistical software (IBM SPSS Statistics Version 25). Analysis of baseline data was carried out using data for all horses for which a baseline questionnaire was completed. Data analyses of changes over the follow-up period included horses for which at least one follow-up questionnaire had been completed.

Continuous data from the questionnaire responses were not normally distributed and are therefore described as medians with interquartile ranges (IQR), and categorical data are described as proportions. For BCS, the mean of three owner-reported scores (neck, ribs, and pelvis) was calculated to provide an overall BCS for each individual questionnaire (described in full in Chapter 5). Owner-reported overall QoL on a scale of 1 (could not be worse) – 10 (could not be better) and ratings for individual QoL domains are reported as medians with IQR. An open-ended question was used to gather information on factors or changes that owners considered to influence their horse's QoL during the follow-up period. Descriptive statistics were used to evaluate changes from baseline over the follow-up period. Pearson

Chi-squared or Fisher's exact tests were used to assess associations between categorical variables. Incidence rates for binary outcomes of i) death/euthanasia, ii) laminitis and iii) infection were calculated and expressed as per 100 horse-years at risk (HYAR) with 95% confidence intervals (CI). Due to the difficulty in accurately defining the date of resolution of an episode of laminitis in recurrent cases, and therefore establishing when an affected horse could be defined as re-entering the population at risk, only the first new episode of laminitis during the follow-up period was included as an outcome of interest. For each of the three outcomes, time at risk was calculated from the date of baseline questionnaire completion, which signalled the date of entry of the horse into the study, until the date on which they were euthanased or died, reported to have a new episode of laminitis, or an infection (e.g. abscess, infected slow healing wound, eye infection). For each analysis, horses that did not have the outcome of interest were censored on the date of the last completed follow-up questionnaire. A Kaplan-Meier estimate of overall survivor function was performed, and log rank (Mantel-Cox) tests were used to compare survival curves between pergolide-treated and non-treated PPID cases. Statistical significance for all analyses was set at $p<0.05$.

Results

Database searches identified a total of 135 PPID cases, of which ten were excluded following review of clinical records as PPID diagnosis or commencement of PPID treatment was prior to 1st January 2014, and three further cases were excluded as results of endocrine assays did not meet inclusion criteria (Figure 1). A further seven cases were excluded as they had not been attended by UoLEP for over two years or owner contact details provided were not up-to-date (Figure 1). Five cases had been euthanased prior to the study start date, and two cases had been sold. Of the remaining 108 cases, 79 owners were contactable and all agreed to participate in the study at the opt-in phone call. As some owners cared for multiple horses (>1), this provided clinical record data for 83.3% of eligible PPID cases (n=90/108).

Retrospective review of clinical records

Clinical records of the 90 PPID cases for which owners gave consent were reviewed, and veterinary follow-up data were available for 88 of these cases during the prospective cohort study period, irrespective of owner questionnaire completion. The remaining two cases did not receive any veterinary visit after the start date for prospective data collection, and were

lost to veterinary follow-up during 2017. Information regarding PPID diagnosis was not available for four cases that had been diagnosed prior to registration with UoLEP. The vast majority of cases were diagnosed with PPID based on elevated basal ACTH (96.5%; n=83/86), with only 3 cases (3.5%) diagnosed based on results of a TRH stimulation test. Forty-four percent of cases (n=38/86) were diagnosed during autumn months (August-October, inclusive, as per months included in laboratory seasonally adjusted reference intervals), with the remainder diagnosed during non-autumn months. Prior to the diagnosis of PPID, 26.7% of cases (n=23/86) had at least one measurement of basal ACTH that was within seasonally adjusted reference intervals (median of 1 previous test; IQR 1-2). The median time between the first normal/equivocal ACTH result and subsequent PPID diagnosis was 2.88 years (IQR 0.95-4.02 years), and laminitis was the most frequent reason for undertaking the initial ACTH measurement (21.7%; n=5/23). Overall, 18.6% of cases (n=16/86) had a history of laminitis prior to any laboratory testing for PPID. An active episode of laminitis was the primary reason for ACTH measurement in 17.0% of cases (n=15/88), and eight of these animals also had a history of previous episodes of laminitis. A few cases (8%; n=7/90) were not treated for PPID following diagnosis, and for pergolide-treated animals, the median recorded starting dose was 1mg/animal/day for both horses and ponies (IQR for ponies 0.5-1mg; range 0.25-1mg; IQR for horses 1-1mg; range 0.25-2mg).

Of the 18 cases that did not have a baseline questionnaire completed, four were euthanased during the prospective data collection period due to colic (n=2) or recurrent laminitis (n=2). There was no association between owner completion of the baseline questionnaire and mortality during the follow-up period (p=0.84) or cases receiving pergolide treatment at the time of diagnosis (p=0.69).

Prospective cohort study population

Subsequently, 84.8% of owners (n=67/79) then completed a baseline questionnaire, providing data for 72 PPID cases that were enrolled in the prospective cohort (Figure 1). Follow-up data were not available for 10 horses as owners were not contactable by telephone and did not respond to emails inviting them to complete follow-up questionnaires online. Comparison between PPID cases that had at least one follow-up (n=62) and those cases with baseline data that did not have at least one completed follow-up questionnaire (n=10), and there were no differences in population medians for age (p=0.48) or height

(p=0.46). Additionally, there were no significant differences in median time since diagnosis (p=0.71), daily pergolide dose (p=0.89) or QoL rating at baseline (p=0.17) between cases with follow-up data and those lost to follow-up at the start of prospective data collection. Additionally, administration of pergolide treatment at the time of baseline data collection was not associated with going on to complete at least one follow-up questionnaire (p=0.09).

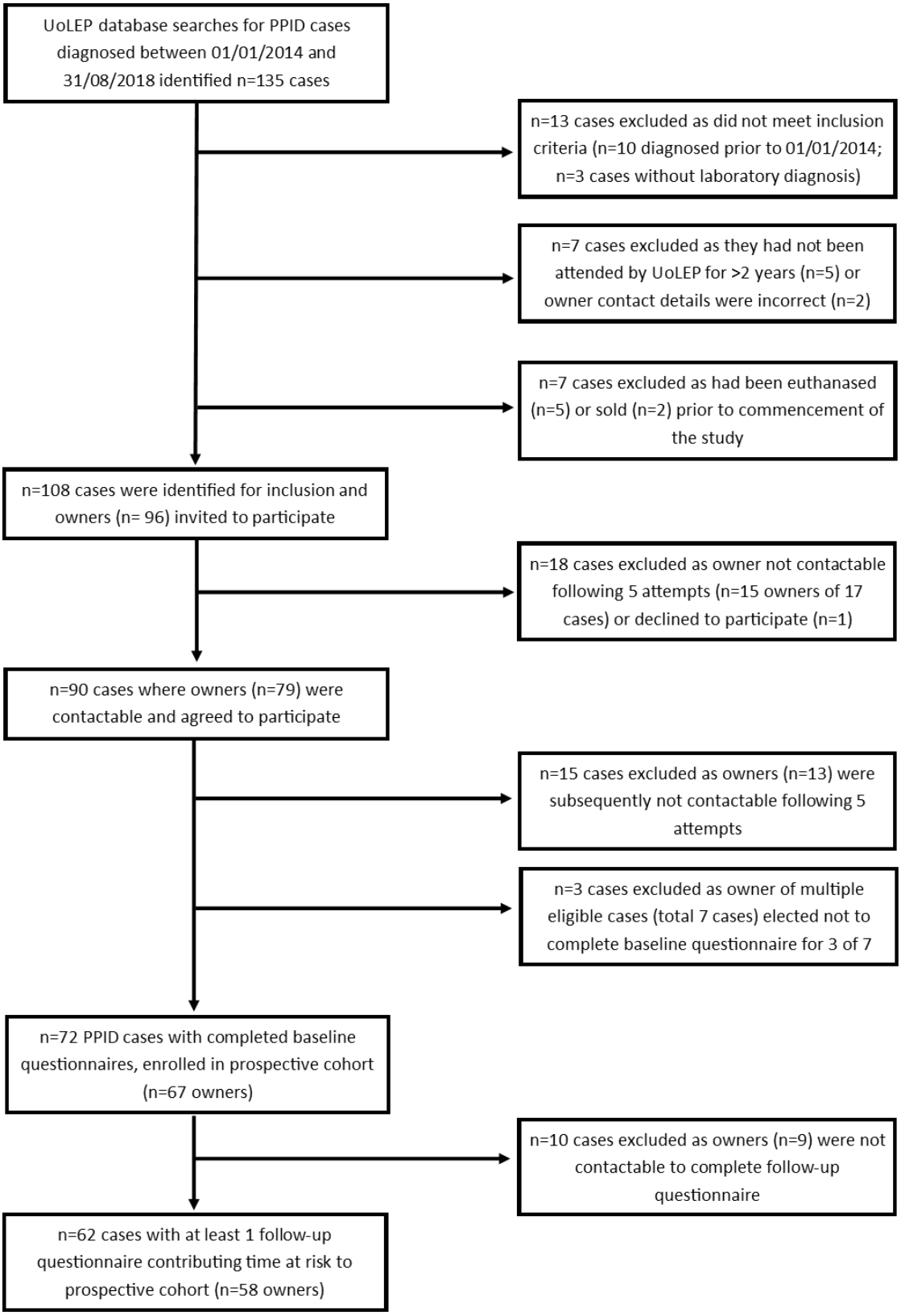


Figure 1: Flowchart demonstrating the process of how cases were recruited to participate in the baseline questionnaire and prospective cohort study.

The remaining 62 horses in the cohort contributed a total of 53.4 HYAR, with a median duration of follow-up of 330.7 days (IQR 247.1-388.9 days). All losses to follow-up between the first and fourth follow-up questionnaires occurred due to unsuccessful telephone contact within five separate attempts (11.3%; n=7). Eleven cases of mortality were reported during the follow-up period, therefore, at the end of the follow-up period 44 horses were alive with complete owner-reported follow-up data.

Demographics and diagnosis

The median age of the population at baseline was 24.5 years (IQR 20.3-27.8 years) and the median duration of ownership was 14.0 years (IQR 7.1-19.0 years). The study population comprised 51.4% (n=37/72) geldings and the remaining 48.6% (n=35/72) were mares. Based solely on height, 58.3% of the cohort (n=42/72) were ponies (≤147.3 cm in height). The most numerous breeds were native/native-cross breeds (43.1%; n=31/72), followed Irish Draught/Irish Draught-cross (15.3%; n=11/72) and Thoroughbred/Thoroughbred-cross (13.9%; n=10/72).

Based on clinical record data, horses had been diagnosed with PPID for a median of 2.1 years (IQR 1.1-3.3 years) prior to baseline data collection. Where date of diagnosis was available, over half of horses (56.5%; n=39/69) were diagnosed during non-autumn months (Nov-Jul), while the remainder were diagnosed during autumn months (Aug-Oct). The median ACTH level reported in clinical records at the time of diagnosis was 93.0pg/ml (IQR 66.6-151.0pg/ml). As expected, the median ACTH level of horses diagnosed in autumn (127.0 pg/ml) was significantly higher compared to those diagnosed during non-autumn months (72.8 pg/ml) ($p<0.001$). When owner-reported ACTH levels were compared to clinical record data, only 14.4% (n=12/69) of owners reported a broadly similar result (within ±20pg/ml).

Treatment and veterinary visits

At baseline, 81.9% (n=59/72) of cases received pergolide treatment, which was administered once daily by the majority of owners (93.2%; n=55/59) and the median dose was 1mg/animal/day (IQR 1-1mg/animal/day). Throughout the follow-up period, pergolide dose remained the same for 78.2% (n=43/55) of cases, decreased in 7.3% (n=4/55), increased in 10.9% (n=6/55) and treatment was stopped in two cases (3.6%). The reason

reported for stopping treatment in both cases was a lack of noticeable beneficial effect by the owner. One previously untreated case started pergolide treatment during the follow-up period. Throughout the follow-up period, 81.8% (n=45/55) of cases remained on once daily dosing. For 9.1% of cases (n=5/55), pergolide administration changed from being split twice daily to given once daily and for 7.3% (n=4/55) dosing changed from once daily to splitting the dose and administering twice daily. The owner of one further case reported changing from daily dosing to administering the same dose every other day (i.e. administered once every 48 hours). Eleven percent of cases (n=8/72) received some form of alternative (n=5) or complementary (n=3 concurrently receiving pergolide) treatment at baseline, most frequently *Vitex Agnus castus* (5.6%; n=4/72) or another herbal supplement marketed for PPID cases (4.2%; n=3/72). During follow-up, one case was reported to receive a new complementary treatment during follow-up while alternative or complementary treatment discontinued in two cases due to lack of noticeable effect.

Owners reported over half of horses (58.1%; n=36/62) to have received ≥1 veterinary visit for PPID monitoring, including an ACTH assay performed, while clinical data showed that 69.4% (n=43/62) had ≥1 veterinary visit including an ACTH assay. A further two owners had monitoring visits but refused a blood test. The median number of ACTH monitoring assays carried out per horse over the follow-up period was 1 (IQR 0-2). Clinical record data showed that 40.3% (n=25/62) had a single ACTH assay performed, while 16.1% (n=10/62) had two. Of those that had one ACTH assay during follow-up, the majority (68.0% n=17/25) were performed during non-autumn months (Nov-July), and of those that had two ACTH assays, most (90.0%; n=9/10) tests were performed during both autumn (Aug-Oct) and non-autumn months.

Based on the seasonally adjusted reference intervals (RI) detailed in the methods section above, 51.4% (n=18/35) of horses had ≥1 ACTH concentrations within seasonally adjusted RIs during the follow-up period. Of the horses that had a single ACTH test during the follow-up period, 48.0% (n=12/25) had a concentration within seasonally adjusted RIs. Of those that had >1 test, 60.0% (n=6/10) had ACTH concentrations that were consistently within RIs, and the remaining four cases had high concentrations that decreased to within seasonally adjusted RI. Of the horses that had elevated or consistently elevated ACTH concentrations, 17.6% (n=3/17) had their pergolide dose increased during the follow-up period.

Owners reported that 44.4% (n=28/62) of horses were vaccinated for either tetanus of equine influenza during the follow-up period, while clinical records showed that 53.2% (n=33/62) were recorded as having been vaccinated.

Description of changes over the follow-up period

Management

Less than half of horses (43.1%; n=31/72) were reportedly in regular ridden exercise at baseline, which consisted of low-level leisure activities such as hacking and schooling for all horses in work. Of those horses in regular ridden exercise at baseline for which follow-up data were available (43.5%; n=27/62), of these 22.2% (n=6/27) were retired during the follow-up period, the level/intensity of work increased for 14.8% (n=4/27) of cases and 14.8% (n=4/27) resumed work after not being exercised for a period of time.

Median turnout hours for each season at baseline and throughout the follow-up period are shown in Figure 2. Unsurprisingly, the amount of forage fed varied with season in over half of cases (54.8%; n=34/62) with owners reporting they decreased the amount during spring and summer months and increased it during autumn and winter months. The amount of forage fed changed for reasons other than season (such as weight management) in 24.2% (n=15/62) and did not change during follow-up in 21.0% (n=13/62) of cases. Half of horses had their bucket feed changed during the follow-up period (50.0%; n=31/62); the most common changes were increasing or decreasing the amount of current feed (58.1% n=18/31), the addition of another feed type (38.7% n=12/31) (additional feeds included concentrates/balancer [33.3% n=4/12] and sugar beet [25.0% n=3/12]) or the addition of a supplement (35.5%; n=11/31) (including pro/pre-biotics [41.7% n=5/12], linseed oil and joint supplements [both 25.0% n=3/12]).

The median overall BCS (calculated as the mean of three owner-reported scores for neck [1-5 scale], ribs and hindquarters regions based [0-5 scale] at baseline was 2.5/5 (IQR 2-3). Throughout the follow-up period, BCS remained constant in 25.8% (n=16/62) of horses, decreased in 30.2% (n=19/62), increased in 17.7% (n=11/62) and fluctuated in 25.8% (n=16/62).

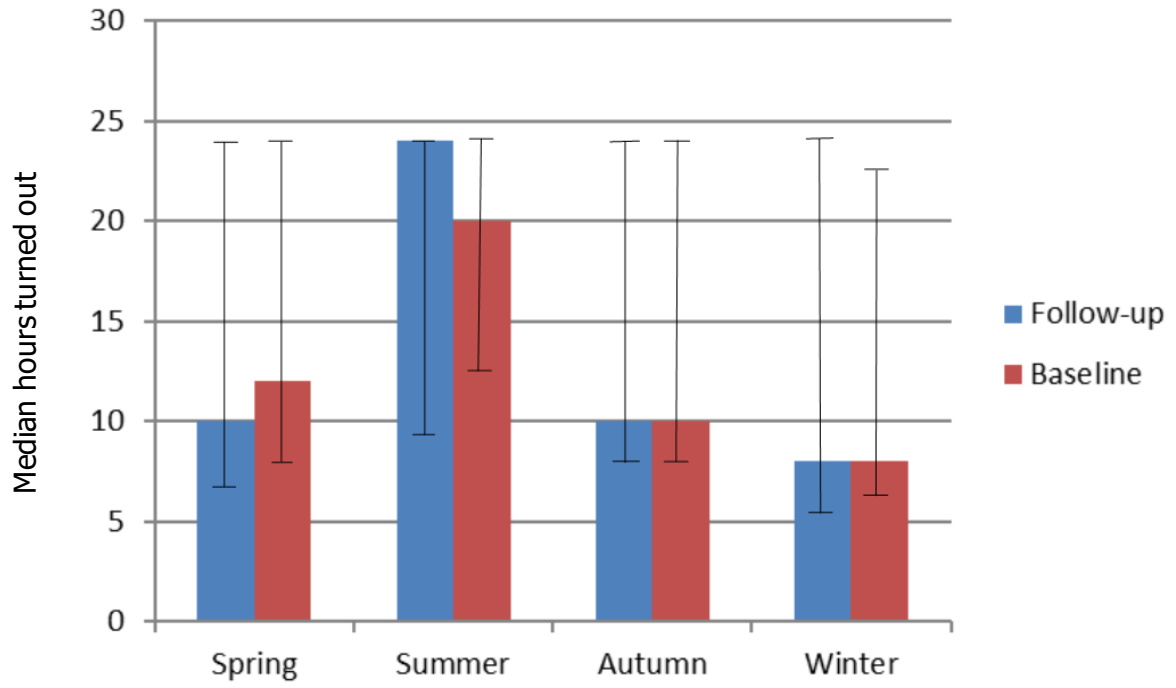


Figure 2: Median owner-reported turnout hours throughout the year at baseline and during the follow-up period with inter quartile ranges.

Quality of life and clinical signs

Median overall QoL rating at baseline was 9/10 (IQR 8-10). Quality of life rating remained unchanged during follow-up in 43.5% (n=27/62) of horses, decreased in 21.0% (n=13/62), increased in 8.1% (n=5/62) and fluctuated in 27.4% (n=17/62). Where QoL fluctuated it was reported to decrease for a period and then increase in 22.6% (n=14/17) and increase for a period and then decreased in 4.8% (n=3/17). The most frequently reported reason for a decrease in overall QoL rating during follow-up was development or worsening of osteoarthritis (14.5%; n=9/13) and all horses that were reported to suffer from laminitis had a decreased QoL during each episode (100%; n=8/8).

Median ratings for appetite and demeanour were both 10/10 (IQR 9-10) at baseline. Demeanour rating remained unchanged during follow-up in 51.6% (n=32/62), decreased in 16.1% (n=10/62), increased in 16.1% (n=10/62) and fluctuated in 16.1% (n=10/62). Appetite rating remained unchanged in 67.7% (n=42/62), decreased in 6.5% (n=4/62), increased in 12.9% (n=8/62) and fluctuated in 12.9% (n=8/62). Median discomfort rating was 0/10 (IQR 0-2). Discomfort rating remained unchanged during follow-up in 48.4%

(n=30/62), decreased in 16.1% (n=10/62), increased in 17.7% (n=11/62) and fluctuated in 17.7% (n=11/62).

The most frequently reported clinical signs at baseline were abnormal coat shedding (26.4%; n=19/72), a history of laminitis (23.6%; n=17/72) and hypertrichosis (22.2%; n=16/72). Changes in clinical signs over the follow-up period are described in Table 1. None of the clinical signs observed were significantly associated with season. Unsurprisingly, abnormal coat shedding was most frequently reported by owners in the spring/summer (22.9%; n=22/62).

Table 1: Clinical signs reported at baseline and throughout the follow-up period in a cohort of 62 horses diagnosed with PPID divided based on whether clinical signs were reported at baseline or not.

Clinical sign	**PPID cases with no clinical signs at baseline** (25.8%; n=16/62)		**PPID cases with ≥1 clinical sign at first follow-up** (74.2%; n=46/62)							
	New clinical signs reported during follow-up period		**Clinical signs observed at baseline**		**Clinical signs resolved during follow-up**		**Clinical signs persisted throughout follow-up**		**Developed new clinical sign during follow-up period**	
	%	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**
None	50.0	8	-	-	-	-	-	-	23.9	11
Active laminitis	6.3	1	2.2	1	2.2	1	-	0	15.2	7
Previous history of laminitis	-	0	32.6	15	-	0	32.6	15	8.7	4
Abnormal fat distribution	-	0	10.9	5	4.3	2	-	0	2.2	1
Pot-bellied appearance	12.5	2	17.4	8	4.3	2	10.9	5	6.5	3
Epaxial muscle wastage	6.3	1	10.9	5	2.2	1	8.7	4	10.9	5

Supraorbital or periorbital fat deposits	6.3	1	21.7	10	4.3	2	8.7	4	2.2	1
Excessive/patchy sweating	-	0	6.5	3	6.5	3	-	0	4.3	2
Polydipsia and polyuria	-	0	6.5	3	2.2	1	-	0	2.2	1
Lethargy/poor performance	-	0	4.3	2	4.3	2	-	0	4.3	2
Recurrent/persistent infection	12.5	2	10.9	5	2.2	1	-	0	21.7	10
Abnormal coat shedding	12.5	2	37.0	17	15.2	7	17.4	8	17.4	8
Curly coat	-	0	4.3	2	2.2	1	2.2	1	6.5	3
Hypertrichosis	18.8	3	28.3	13	13.0	6	8.7	4	8.7	4
Changes to coat colour/texture	6.3	1	17.4	8	10.9	5	2.2	1	13.0	6

Mortality and disease incidence rates

During the follow-up period, 17.7% (n=11/62) of horses died, representing an overall mortality rate of 20.6 deaths per 100 HYAR (95% CI 10.3-36.9). A Kaplan-Meier estimation of overall survival of the cohort is shown in Figure 3, and estimated mean survival time was 428.0 days (95% CI 397.2-458.7 days). All cases of mortality were euthanased by a veterinary surgeon. The most common reason for euthanasia was colic (63.6%; n=7/11), and the remaining cases were euthanased due to recurrent laminitis, injury, severe infection and behavioural issues (each 9.0%; n=1). Owners of three cases reported that the fact their horse had PPID influenced their decision to have them euthanased, cause of death in these cases were acute illness, laminitis and behavioural issues. A further five horses were reported to have a decreased QoL prior to euthanasia, cause of death in these cases were colic (n=2), acute illness (n=1), laminitis (n=1) and neurological signs (n=1). There was no significant difference in the probability of survival between pergolide-treated cases (n=54) and those not receiving pergolide (n=8) (p=0.19).

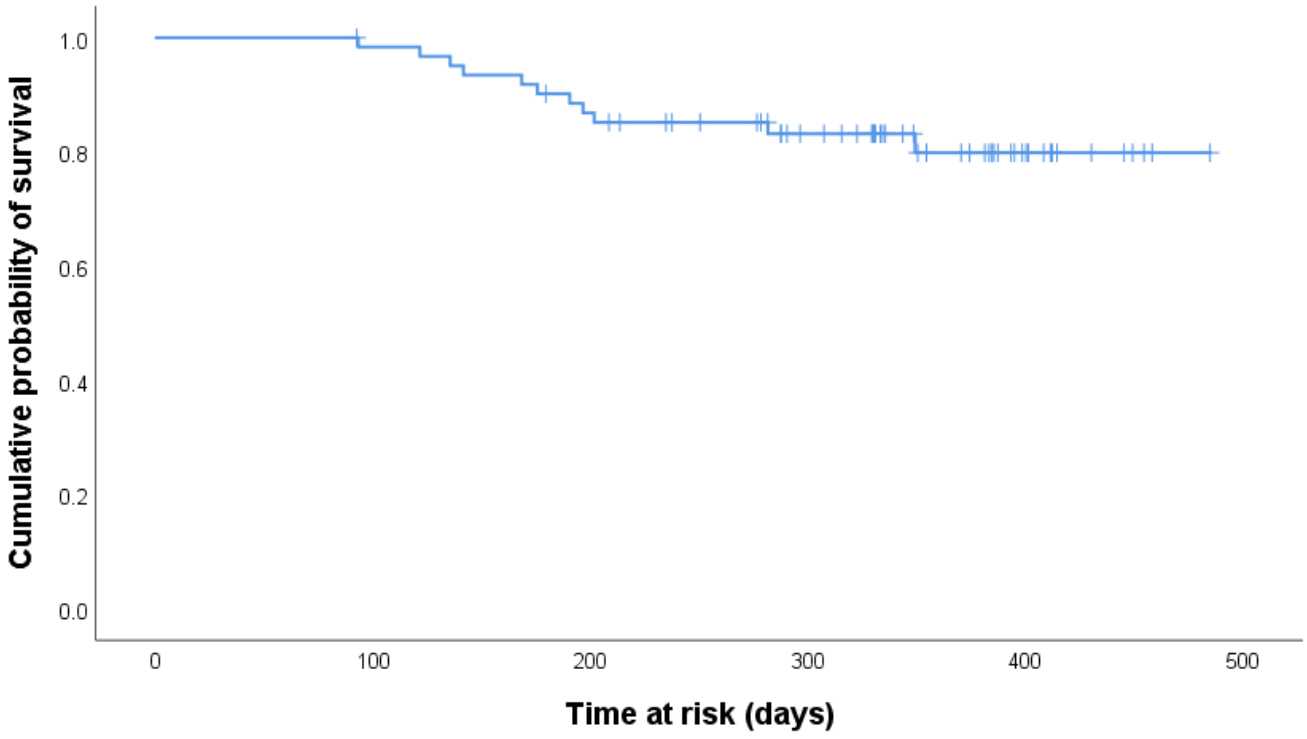


Figure 3: Kaplan-Meier estimate of overall survivor function for a cohort of diagnosed PPID cases registered with the University of Liverpool Equine Veterinary Practice (n=62).

New episodes of laminitis during the follow-up period were reported in 12.9% (n=8/62) of horses, of these; six were veterinary-diagnosed, and four horses had a previous history of laminitis. Two cases were reported to have had multiple episodes of laminitis during the follow-up period, with only time at risk to the date of the first episode included in incidence and survival analysis. Enrolled animals contributed a total of 51.0 HYAR for laminitis, and the incidence of laminitis was estimated to be 15.7 laminitis episodes per 100 HYAR (95% CI 6.8-30.9 first new episodes per 100 HYAR). Six of the eight animals with at least one episode of laminitis during the follow-up period were receiving pergolide treatment, including the two cases with multiple laminitis episodes. The probability of not having an episode of laminitis was not significantly different between pergolide-treated cases (n=54) and those not receiving pergolide (n=8) (p=0.15).

During follow-up, 19.4% (n=12/62) of horses were reported to have had an occurrence of infection, of which nine were veterinary-diagnosed and two had a previous history of recurrent infections. Types of infection reported were subsolar abscess (50.0%; n=6/12), ocular infection (41.7%; n=5/12) and skin infection (8.3%; n=1/12). Enrolled animals contributed a total of 48.3 HYAR for infections, and the incidence of infection was estimated to be 24.9 events of infection per 100 HYAR (95% CI 12.8-43.4 episodes of infection per 100 HYAR). Eleven of the 12 horses with at least one episode of infection during the follow-up period were receiving pergolide treatment. There was no difference in the probability of not having an episode of infection between pergolide-treated cases (n=54) and those not receiving pergolide (n=8) (p=0.58).

Discussion

The results presented here describe the changes in management, treatment, health and QoL in a field-based prospective cohort of horses with PPID, and provides useful information regarding rates of mortality and morbidity in a selected population of PPID cases. Many horses developed new morbidities during the follow-up period and overall mortality rate was 20.6 deaths per 100 HYAR. QoL was excellent at baseline but decreased or fluctuated in half the horses, related to the development of comorbidities.

Overall, the loss to follow-up was slightly higher than anticipated. A high level of loss to follow-up can lead to selection bias (Howe et al., 2016). Historically, a follow-up rate of 60% was considered good (Babbie, 1973), while a more recent study utilised simulated data sets

and reported that loss to follow-up can range for 5-60% without significant biases being observed (Kristman et al., 2004). The use of telephone recruitment and questionnaires resulted in good recruitment and retention rates, with a low loss to follow-up once participants were enrolled in the study, therefore the risk of selection bias is likely to be low. This methodology is also likely to have limited the risk of response bias and item omission, which can be a limitation of online or paper surveys (Paulhus, 1991; Fleming and Bowden, 2009;). Moreover, the single veterinary practice, convenience sampling used to recruit participants and relatively small number of cases available at final follow-up means the population may not be entirely representative of the general population. However, the demographic characteristics of the study population were comparable to the larger cross-section described in Chapter 5. Results derived from a wider population across multiple practices may be likely to differ. However, since pergolide is the only licensed medical therapy for PPID in the UK, it is probable that treatment recommendations would be broadly similar between different practices. Sampling cases from a single veterinary practice meant that diagnostic and treatment strategies undertaken are likely to have been similar among participants, and this similarity enabled the investigation of owner management changes, although it may reduce generalisability to other practice populations. Additionally, the use of both owner-reported data and clinical records allowed gaps to be filled where data was deficient or corrected where incorrectly reported.

Follow-up questionnaires were conducted at regular intervals throughout the study, and the timing of each follow-up questionnaire would have been likely to influence owner responses. The QoL rating given would have been influenced by the clinical signs of PPID the horse was exhibiting at the time of follow-up. The degree to which owners consider clinical improvement synonymously with improvement in QoL has not been investigated to date and warrants further research. Owner ratings of QoL are also likely to be influenced by the presence or absence of other health problems. For example, during an episode of lameness, the owner QoL rating may be lower than several weeks prior to observation of lameness or after resolution. However, this study does uniquely demonstrate changes over time in horses with PPID, and highlights the important effect of comorbidities on overall QoL and owner perceptions of levels of discomfort.

The mortality rate reported for the general geriatric population in the UK was 11.1 per 100 HYAR (Ireland et al., 2011d), while a rate of 9.4 per 100 HYAR has been reported for the

geriatric population in Australia (McGowan, 2009). More recently, the incidence of euthanasia was 7.3 per 100 HYAR in a cohort of horses, with a median age of 15 years, recruited to a large laminitis study in Great Britain (Pollard et al., 2020). As PPID has been associated with an increased risk of mortality (Welsh et al., 2016) it is unsurprising that the incidence of mortality reported here is higher compared to these studies. The slightly older population reported here may also have influenced the incidence of mortality, as a previous study reported that the rate of mortality increased with increasing age, from 6.5 per 100 HYAR for horses aged 15–19 years, to 35.2 per 100 HYAR aged >30 years (Ireland et al., 2011d). PPID was not reported as a cause of death in any cases and only three owners reported that the fact their horse had PPID influenced their decision to euthanase. The most frequently reported reason for mortality reported here was colic. Comparably, colic was also one of the most frequent reasons for euthanasia in a large field-based study (Pollard et al., 2020) and in the general geriatric population (Ireland et al., 2011d).

The majority of horses were reported by owners to have at least one clinical sign of PPID at baseline and also went on to develop at least one new clinical sign during the study. Of those horses reported to be exhibiting no clinical signs at the first follow-up, half remained free of clinical signs. Owners have been shown to underreport clinical signs of disease (Ireland et al., 2012a), however, in this study owners were aware their horse had PPID and therefore may monitor them more closely for clinical signs. The most frequently reported new clinical signs were; abnormal coat shedding and recurrent infections, while the clinical sign most frequently reported to resolve was also abnormal coat shedding. Coat changes were indicated in both groups and are well documented clinical signs of PPID (Schott, 2002; Innerå et al., 2013). These abnormalities are likely to be easiest for owners to notice and monitor, while also being most likely to resolve as they may not be observed during some seasons. It is therefore unsurprising they are most frequently reported. These data were predominantly based on owner-reported information as veterinary records often did not provide enough detail to enable the reporting of individual clinical signs. This highlights the importance of keeping correct and detailed clinical records that allow accurate data extraction for use in research.

The incidence of laminitis reported in this cohort was higher than reported in previous studies. An Australian study of horses attending Pony Club events reported an incidence of laminitis within horse and pony groups separately: the pony group sampled was 6.5 cases

per 100 HYAR, while the incidence in horses was 0.55 cases per 100 HYAR (Potter et al., 2017). A large prospective cohort reported an overall incidence of owner-reported laminitis of 9.6 per 100 HYAR for single episodes (Pollard et al., 2019). The higher incidence reported in this study may be due to the increased risk of laminitis in geriatric horses and horses diagnosed with PPID. Increasing age has been associated with an increased risk of laminitis (Alford et al., 2001) and laminitis is one of the most frequently reported clinical signs in PPID cases with an overall prevalence of 48.9% (Ireland and McGowan, 2018). The incidence of infections was higher than the overall incidence of owner-reported laminitis. Despite being a well-documented clinical sign of PPID, the prevalence of concurrent or recurrent infections is rarely documented (Ireland and McGowan 2018). To the authors knowledge this study is the first to report the incidence of infections in horses with PPID, which provides a valuable benchmark for comparison in future research.

Despite the frequency of morbidities, both the general geriatric population (Ireland et al., 2011c) and horses diagnosed with PPID have been reported to have a very good owner-reported QoL (Chapter 5). In this study, cases were reported to have a very good owner-reported QoL at baseline. However, this rating changed in the majority of horses, with QoL reported to decrease over the follow-up period for over 20% of horses. Similarly discomfort ratings increased in nearly 18% of horses during the follow-up period. The main reason given by owners for a reduction in QoL was osteoarthritis and not related to PPID. Osteoarthritis is also age-associated and has been reported to be a common comorbidity in geriatric horses and PPID cases (Ireland et al., 2011d; Welsh et al., 2016). Owners may be more likely to notice the lameness and discomfort related to osteoarthritis, compared to some of the subtler clinical signs of PPID, and therefore associate it with a reduction in QoL. Unsurprisingly, all horses that were reported to suffer from laminitis had a decreased QoL during each episode. Decreased QoL has been reported to be associated with mortality in the general geriatric population (Ireland et al., 2011d), however it was out of the scope of this study to investigate factors associated with mortality. The occurrence of morbidities and risk of mortality were not affected by treatment status. The majority of cases were receiving pergolide treatment, with only a few owners choosing not to administer treatment or to use an alternative treatment. This is similar to the findings in Chapter 5 and a recent Australian study where 88.3% of cases were receiving pergolide treatment (Horn et al., 2019). For those cases which received pergolide treatment, the median dose was also comparable to other studies (Chapter 4; Chapter 5; Horn et al., 2019). Pergolide treatment was not

significantly associated with reduced incidence of mortality, laminitis or infection. This differs from the finding of Horn et al, (2019) which reported that pergolide treatment was positively associated with survival. However, both studies had relatively few horses that were not treated with pergolide, and the power of the current study is likely to be low for this reason, suggesting further work in this area is required.

The majority of cases were maintained on the same dose of pergolide treatment throughout the follow-up period. However, nearly a third of horses did not receive a PPID veterinary assessment, including ACTH monitoring, during the follow-up period. Therefore, for many horses pergolide dose may have remained unchanged because of insufficient monitoring, rather than being due to satisfactory control of the disease. Additionally, owners of several cases reported decreasing or stopping treatment, suggesting non-compliance with veterinary recommended treatment (Steel et al., 2020; Hague et al., 2021). However, of those horses receiving pergolide treatment that had ≥1 ACTH assay performed during follow-up, just over half were within seasonally adjusted RIs. This is much higher than reported in a large retrospective study (28%; Rendle et al., 2013) and a recent cohort (34.7%; Horn et al., 2020). Rendle et al., (2013) also reported that duration of treatment was positively associated with treatment response and that UK native breeds were more likely to return to within the reference interval. Therefore, the time since diagnosis and over-representation of native breeds in the population described here, may have contributed to the increased proportion of cases with ACTH levels within RIs.

When owner-reported ACTH levels were compared with clinical records at diagnosis, there was a lack of accurate recall. This is unsurprising as horses were diagnosed a median of 2.1 years previously, which may have increased the chance of recall bias. However, owners also under-reported monitoring veterinary visits and administration of routine vaccination. This demonstrates that recall bias can be an issue for owner-reported data even within a relatively short timeframe, and highlights the benefit of utilising clinical record data alongside owner-reported information. Clinical records demonstrated just under half of horses were not up-to-date with their vaccinations. This is in keeping with the fact that the frequency of vaccination reportedly reduces with increasing horse age and retirement (Mellor et al., 2001). However, the proportion reported to be vaccinated during follow-up in this study (53.2%) was lower than the reported in the general geriatric population (Ireland

et al., 2011b), where the majority of horses were regularly vaccinated for tetanus (82.8%) and equine influenza (66.3%).

Retirement from exercise, turnout and BCS varied throughout the study based on routine seasonal changes as well as morbidities. Throughout follow-up, turnout hours during spring and autumn differed slightly from those reported by owners at baseline. Cases were reported to spend more time turned out during spring and more less turned out during the summer months. This may have been due to the phrasing of questions in the separate questionnaires. At baseline, owners were asked to report how long their horse was 'usually turned out for', compared to current hours during follow-up which is likely to have led to differences in response. Additionally, a small amount of variation may have be the result of specific weather conditions at the time of follow-up, which may have affected the amount of time horses spend turned out. During follow-up, owner-reported BCS changed in the majority of horses and was most frequently reported to decrease. Weight loss has been reported as a clinical sign of PPID (Ireland and McGowan, 2018) and has been associated with risk of mortality in geriatric horses (Ireland et al., 2011d) and horses with PPID (Horn et al., 2019). The overall BCS reported here was lower than for the general geriatric population, where the majority of horses (80.9%) were reported to be in good body condition (Ireland et al., 2011b). However, the change in BCS reported here may have been due to owner misinterpretation of clinical signs such as muscle loss and fat redistribution rather than true weight loss. A reasonable amount of horses were still in regular exercise despite their age. However, the number of horses in work was lower than reported in the geriatric population. Ireland et al (2011a) reported that 62% of horses were used for hacking/pleasure while over a quarter were still competing. The reduction in exercise reported here is perhaps unsurprisingly, as the amount of exercise was reported to decrease in intensity with increasing age (Ireland et al., 2011a).

Conclusion

In summary, the same treatment regime for most horses remained unchanged during the follow-up period, despite the majority of owners reporting the development of new clinical signs of PPID. Horses were perceived to have a very good QoL but this changed during follow-up in the majority of cases, with laminitis and osteoarthritis having the biggest detrimental impact on QoL rating. Many horses did not receive the recommended level of PPID monitoring, but of those that did, ACTH levels were predominantly well controlled.

Treatment regime did not significantly mortality rate or the incidence of laminitis or infection. Incidence of mortality was higher than in the general geriatric population, however none of the reasons for mortality were related to PPID. The prospective changes described in this cohort provide useful additional information on the management, treatment and clinical factors observed in horses with PPID as well as the long-term practices undertaken by owners. This will be useful for veterinary surgeons when advising on the long-term management and prognosis of horses with PPID, and for furthering owner education regarding monitoring cases for the development or progression of clinical signs.

Appendices for Chapter 6

Appendix 1 – Invitation letter and project information sent to participants by University of Liverpool Equine Practice

Dear [client's name],

The University of Liverpool is conducting a survey of owners of horses and ponies diagnosed with Pituitary Pars Intermedia Dysfunction (PPID, also known as Cushing's syndrome).

In our previous research, consultation with vets and owners with experience of PPID has identified important research questions, which you as carers want answered. One of top ten most important research priorities was to investigate the most effective management and treatment strategies for horses and ponies with PPID. We are writing to invite you to participate in a research study we hope will go some way to addressing this important area of research.

The purpose of this study is to improve our knowledge and understanding of current management and treatment approaches undertaken and to develop healthcare plans to improve the quality of life of horses and ponies with PPID. However, this research could not be done without your help. The answers you provide about your horse or pony can help make a real difference to the health of horses and ponies with PPID.

Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the enclosed study information sheet carefully and feel free to ask us if you would like more information or if there is anything that you do not understand (using the contact details provided below).

The initial survey will be conducted by telephone and consists of questions about your horse's routine, feed, general health and, where applicable, treatment for PPID. We will also ask you to complete three short follow-up questionnaires to document any changes over time. Your privacy is, of course, of the utmost importance to us and if you decide to

take part, all responses are completely confidential and will only be used for the purposes of this survey.

We hope you will be able to participate, however your involvement is entirely voluntary, if you wish to **opt out** please email rebecca.tatum@liverpool.ac.uk or telephone Becky on 01638 751000 extension 1241. If we do **not** hear from you by xx 2018 *(three weeks after the date the letters are sent)*, the University of Liverpool researcher, Becky Tatum, will contact you by telephone.

Thank you in anticipation of your support.

Yours sincerely,

Becky Tatum, PhD Student, University of Liverpool

Dr Joanne Ireland, PhD Supervisor, University of Liverpool

Professor Catherine McGowan, PhD supervisor, University of Liverpool

Information Sheet

Optimising Care for Horses and Ponies with PPID

You are being invited to participate in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and feel free to ask us if you would like more information or if there is anything that you do not understand. Please also feel free to discuss this with your friends, relatives or veterinary surgeon if you wish. We would like to stress that are free to opt out of participation and should only agree to take part if you want to.

What is the purpose of the study?

The purpose of this study is to investigate what management and treatment factors are most likely to improve quality of life for horses and ponies with PPID. Quality of life is a way of measuring your horse/pony's overall health, wellbeing and happiness.

Why have I been chosen to take part?

You have been chosen to take part in this study because your horse/pony has been diagnosed with PPID (based on a laboratory test result where the hormone ACTH was above the normal reference range for the time of year the sample was taken). If this information is no longer correct, please inform us of any change in circumstances using the contact details below. All owners registered with the University of Liverpool Leahurst Equine Practice whose horse/pony has been diagnosed with PPID in the past three years are being invited to participate in this study. All horses/ponies diagnosed with PPID are eligible to be included in this study, whether or not they are currently receiving any medical treatment for the condition.

About the study

If you agree to take part in the study, we will ask you to take part in a telephone questionnaire, which will take around 20 minutes to complete. You will be asked questions about your horse/pony's

routine, daily care, exercise, health, any medication or other treatments they receive and how you perceive their quality of life. This will be followed by three shorter follow-up questionnaires at three month intervals to document any changes over time. Your responsibility as a participant of this study will be to answer all questions honestly and with your own opinion. We will also use your horse/pony's veterinary records from the Leahurst Equine Practice in order to help us understand the associations between PPID laboratory test results (measuring ACTH), as a potential indicator of disease severity, and health and quality of life of horses/ponies with PPID.

Participating in this study will not interfere with your horse/pony's management, routine preventive healthcare, treatment for PPID or treatment of any other conditions. However, if you chose to make any changes to your horse/pony's management throughout the study, such as feeding, exercise regime, field turnout or stabling and any healthcare you provide such as worming or vaccinations, we would ask you to record these changes and let us know, either directly using the contact details provided, or during a follow-up telephone questionnaire.

The data we collect will be anonymised with no information that could identify you or your horse. It will be kept confidential and stored securely on University password protected computers, it will be used for this specific study only and only the study research team at the University of Liverpool will have access to the data. You or your horse/pony will not be identifiable from the results.

Why is this study important?

Pituitary *pars intermedia* dysfunction is the most common hormonal disorder of older horses/ponies. Previously we have engaged with vets and owners (with experience of PPID) to prioritise areas of research they consider to be important. Establishing which management (including aspects such as dietary and turnout) and treatment strategies are most effective were areas ranked in the top 10 research priorities. The information provided in this study will help us to better understand current management and treatment approaches undertaken for horses/ponies with PPID. The aim is to have more information about management and treatment options for veterinary surgeons and owners in order to improve the quality of life of horses and ponies with PPID.

Are there any risks to myself or my horse/pony?

As we are only using telephone questionnaires to collect data we do not anticipate any disadvantages or risks associated with your or your horse/pony's participation.

What happens if I want to stop taking part?

We would like to stress that your participation is voluntary. As a participant if you wish to end your participation at any point during the phone call or duration of the study you are free to do so. If you decide to withdraw your responses during or at the end of the interview, and if you request it, we will destroy any data regarding yourself or your horse from the study.

Contact details

If you have any questions or for further information please contact:

Becky Tatum

Email: rebecca.tatum@liverpool.ac.uk

Telephone: 01638 751000 Ext. 1241

If you are unhappy, or if there is a problem, please feel free to let us know by contacting Becky Tatum or Jo Ireland and we will try to help. If you remain unhappy or have a complaint which you feel you cannot come to us with then you should contact the Research Governance Officer on 0151 794 8290 (ethics@liv.ac.uk). When contacting the Research Governance Officer, please provide details of the name or description of the study (so that it can be identified), the researcher(s) involved, and the details of the complaint you wish to make.

Thank you for taking the time to read this.

Becky Tatum

The contact details of lead Researcher (Principal Investigator) are: [Contact: Dr Joanne Ireland, Leahurst Equine Practice, Leahurst Campus, University of Liverpool, Neston, Wirral, CH64 7TE, email: joanne.ireland@liverpool.ac.uk]. If there are any problems, please let us know and we will try to help.

Appendix 2 – Follow-up questionnaire

Optimising Care for PPID Horses and Ponies - Follow-Up Questionnaire

As part of our study into Pituitary Pars Intermedia Dysfunction (PPID, also known as Cushing's syndrome), we are asking participants to complete short follow up questionnaires every three months. This will help us to document any changes to your horse's health, management and treatment throughout the study.

Unless otherwise stated, the following questions all refer to the time since your last questionnaire, approximately 3 months ago.

This questionnaire will take around 10 minutes to complete.

If you have more than one horse enrolled on the study, please complete one questionnaire per horse.

The term 'horse' is used to refer to your horse or pony with PPID.

All responses are completely confidential.

1 Owner name

2 Horse name

3 Is your horse still in your care?

- [ ] Yes
- [ ] No, on loan
- [ ] No, sold
- [ ] No, died or put to sleep
- [ ] Other (Please Specify)

4 Does your horse currently receive treatment with pergolide (Prascend)?

☐ Yes ☐ No

5 If you answered yes to Q4, please indicate the dose of pergolide (Prascend) your horse currently recieves (e.g. number of tables and how many times per day)

Number of tablets

Number of times per day

6 Has the dose of pergolide (Prascend) your horse receives changed since the last questionnaire?

☐ No, not changed ☐ Yes, increased ☐ Yes, decreased
☐ Yes, stopped ☐ Not applicable, my horse has never received pergolide

If you answered yes to Q6, please indicate the reasons for stopping or changing the dose of pergolide (Prascend).

7 If you answered yes to Q6, did you consult your vet before making changes to the dose?

☐ Yes ☐ No
☐

Other (Please Specify)

8 Does your horse currently receive any alternative treatments for PPID?

☐ No ☐ Agnus castus/chaste berry ☐ Homeopathy
☐ Herbal supplements (e.g. Cush Aid, Cush-X) ☐ Magnesium ☐ Antioxidants (e.g. vitamins E and C)
☐

Other (Please Specify)

9 Has this changed since the last questionnaire?

☐ Yes ☐ No

If yes, please specify the reason for any change

_____

10 Have there been any changes to your horse's turnout/stabling routine since the last questionnaire? (e.g. stabled more due to change in weather conditions)

☐ Yes ☐ No

☐

If yes please specify

11 On average, how many hours per day does your horse currently spend turned out?

_____

12 Has your horse's hard feed/bucket feed changed since the last questionnaire (including any supplements he/she receives)?

☐ Yes ☐ No

☐

If yes please specify

13 Has the amount of forage your horse receives changed since the last questionnaire?

☐ No, not changed ☐ Yes, increased ☐ Yes, decreased

☐ Yes, no longer fed forage ☐ Not applicable, not fed forage (grass only)

14 On average, has there been any change to the number of days a week you exercise your horse?

- [ ] No, not changed
- [ ] Yes, increased
- [ ] Yes, decreased
- [ ] Yes, no longer ridden
- [ ] Not ridden/retired
- [ ]
- [ ] Other (Please Specify)

15 Has there been any change to the intensity of exercise your horse receives?

- [ ] No, not changed
- [ ] Yes, increased
- [ ] Yes, decreased
- [ ] Yes, no longer ridden

If your horse is not ridden, retired or no longer ridden please go to section 5 on the next page.

16 Have there been any changes to the discipline you normally use your horse for since the last questionnaire?

- [ ] Yes
- [ ] No
- [ ]
- [ ] If yes please specify

17 On average, has there been any change to how long you exercise your horse each time they are ridden?

- [ ] No change
- [ ] Yes, increased
- [ ] Yes, decreased

18 Do you think your horse's body condition has changed since the last questionnaire (approximately 3 months ago)?

- [ ] Yes
- [ ] No

19 Which option best describes the condition of your horse's neck?

- [ ] No visible crest
- [ ] Visible crest but fat is distributed evenly
- [ ] Crest thickened with fat deposited more in the middle
- [ ] Crest is enlarged/thickened
- [ ] Crest is extremely large and can droop to one side

20 Which option best describes the condition of your horse's ribs/stomach?

- [ ] Ribs easily visible
- [ ] Ribs just visible
- [ ] Pot belly (carrying weight low with ribs visible)
- [ ] Ribs covered
- [ ] Ribs well covered
- [ ] Ribs very well covered

21 Which option best describes the condition of your horse's hind quarters?

- [ ] Prominent pelvis and sunken rump
- [ ] Flat rump with little fat
- [ ] Rounded rump evenly covered with fat
- [ ] Rounded rump with uneven fat distribution
- [ ] Very rounded rump with gutter along to base of the tail
- [ ] Heart shaped rump with fat pads and very deep gutter to the base of the tail

22 How would you rate you horse's current demeanor (the way they look, behave and their attitude)?

0 1 2 3 4 5 6 7 8 9 10

Could not be worse — Could not be better

---

23 How would you rate your horse's current appetite?

0 1 2 3 4 5 6 7 8 9 10

My horse has a poor appetite and does not eat enough — My horse has a good appetite and always wants to eat

---

24 How would you rate the level of discomfort your horse is currently experiencing?

0 1 2 3 4 5 6 7 8 9 10

No discomfort — Serious discomfort (e.g. acute laminitis)

---

25 Do you feel that PPID currently affects your horse's normal daily activities?

0 1 2 3 4 5 6 7 8 9 10

Not at all — An extreme amount

---

26 How would you rate your horse's current overall quality of life?

0	1	2	3	4	5	6	7	8	9	10
Could not be worse										Could not be better

27 Are there any factors or changes which have influenced your horse's quality of life since the last questionnaire?

28 If you use faecal worm egg counts, when did your horse last have one done (dd/mm/yy)? (If you do not use faecal worm egg counts please go question 31 below)

29 Do you think your horse is currently showing any clinical signs (symptoms) of PPID?

- [ ] None
- [ ] Active laminitis
- [ ] Hoof changes from previous history of laminitis
- [ ] Abnormal fat distribution (e.g. fat pads around the neck or hind quarters)
- [ ] Pot belly
- [ ] Wasted/loss of muscle over topline
- [ ] Fat pads around the eyes/puffy lower eye lids
- [ ] Excessive/patchy sweating
- [ ] Increased drinking and urination
- [ ] Lethargy/poor performance
- [ ] Recurrent infections (e.g. foot abscesses, sinusitis)
- [ ] Abnormal coat shedding
- [ ] Curly coat
- [ ] Overgrown coat
- [ ] Changes to coat colour/texture
- [ ]

Other (Please Specify)

30 If you can remember the result of your horse's most recent faecal worm egg count please indicate it below.

The following questions are about your horse's routine health care.

31 If your horse was wormed, what were they wormed with?

- [ ] Equest
- [ ] Equest Pramox
- [ ] Equitape
- [ ] Eqvalan
- [ ] Noromectin
- [ ] Panacur
- [ ] Strongoid-P
- [ ] Not applicable
- [ ]

Other (Please Specify)

32 When was your horse last wormed (dd/mm/yy)?

33 When were your horse's teeth last checked? (dd/mm/yy)

34 Were there any problems with your horse's teeth?

- [ ] Yes
- [ ] No
- [ ]

If yes please specify

35 When was your horse last shod or had his/her feet trimmed? (dd/mm/yy)

36 Were there any problems with your horse's feet?

- [ ] Yes
- [ ] No
- [ ]

If yes please specify

37 Has your horse had a routine vaccination visit or check up from your vet since the last questionnaire?

☐ Yes ☐ No

38 Has your horse had a routine PPID visit or check up (including a blood sample) from your vet since the last questionnaire?

☐ Yes ☐ No

☐

Other (Please Specify)

39 Has your horse had any health problems since the last questionnaire?

☐ Yes ☐ No

If your horse has not had any health problems since the last questionnaire, please go to section 9 on the next page.

40 What problem(s) did/does your horse have?

41 What symptoms or signs did you notice?

42 Has your horse had any other problems since the last questionnaire?

☐ Yes ☐ No

43 If yes, please provide details of each problem

44 Did any of the health problems affect your horse's appetite?

- [ ] No, didn't affect appetite
- [ ] Yes, increased
- [ ] Yes, decreased
- [ ] Yes, completely off food

45 What kind of action was required?

- [ ] Vet attendance
- [ ] Vet advice only (e.g. over the phone)
- [ ] Treatment/care by you (the owner)
- [ ] No action

46 If vet attendance was required, how many vet visits were needed?

47 What procedures were carried out?

- [ ] None
- [ ] Routine exam
- [ ] Blood sample(s)
- [ ] Lameness exam
- [ ] Lameness work up including nerve blocks
- [ ] X-ray
- [ ] Rectal exam
- [ ] Endoscopy
- [ ] MRI scan
- [ ] Biopsy
- [ ] Abdominal surgery
- [ ] Orthopaedic surgery
- [ ] Other surgery
- [ ] Sarcoid removal
- [ ]

Other (Please Specify)

48 Did your horse receive any medical treatment?

- [ ] None
- [ ] Injectable pain killer/anti-inflammatory (e.g. Bute)
- [ ] Injectable steroids
- [ ] Injectable antibiotics
- [ ] Other medication
- [ ] Joint medication
- [ ] Anti-spasmodic (e.g. Buscopan)
- [ ] Oral pain killer/anti-inflammatory (e.g. Bute)
- [ ] Wound Care/Dressing
- [ ] Oral steroids
- [ ] Oral antibiotics
- [ ] Skin treatment/cream
- [ ] Inhaler
- [ ]

Other (Please Specify)

49 Did your horse require any other treatment?

- [ ] None
- [ ] Physio/complementary therapy
- [ ] Stitches/staples
- [ ] Hot/cold hydrotherapy
- [ ] Inhand walking/controlled exercise
- [ ] Box rest
- [ ] Poulticing
- [ ] Bandaging
- [ ] Restricted turnout
- [ ] Remedial farriery (e.g. heart bar shoes)
- [ ]

Other (Please Specify)

50 Was your horse referred to a specialist equine hospital (e.g. Leahurst) for any of their treatment?

- [ ] Yes
- [ ] No

51 Has treatment for the condition(s) been completed?

- [ ] Yes
- [ ] No
- [ ] Ongoing
- [ ]

Other (Please Specify)

48 Did your horse receive any medical treatment?

- [ ] None
- [ ] Injectable pain killer/anti-inflammatory (e.g. Bute)
- [ ] Injectable steroids
- [ ] Injectable antibiotics
- [ ] Other medication
- [ ] Joint medication
- [ ] Anti-spasmodic (e.g. Buscopan)
- [ ] Oral pain killer/anti-inflammatory (e.g. Bute)
- [ ] Wound Care/Dressing
- [ ] Oral steroids
- [ ] Oral antibiotics
- [ ] Skin treatment/cream
- [ ] Inhaler
- [ ]

Other (Please Specify)

49 Did your horse require any other treatment?

- [ ] None
- [ ] Physio/complementary therapy
- [ ] Stitches/staples
- [ ] Hot/cold hydrotherapy
- [ ] Inhand walking/controlled exercise
- [ ] Box rest
- [ ] Poulticing
- [ ] Bandaging
- [ ] Restricted turnout
- [ ] Remedial farriery (e.g. heart bar shoes)
- [ ]

Other (Please Specify)

50 Was your horse referred to a specialist equine hospital (e.g. Leahurst) for any of their treatment?

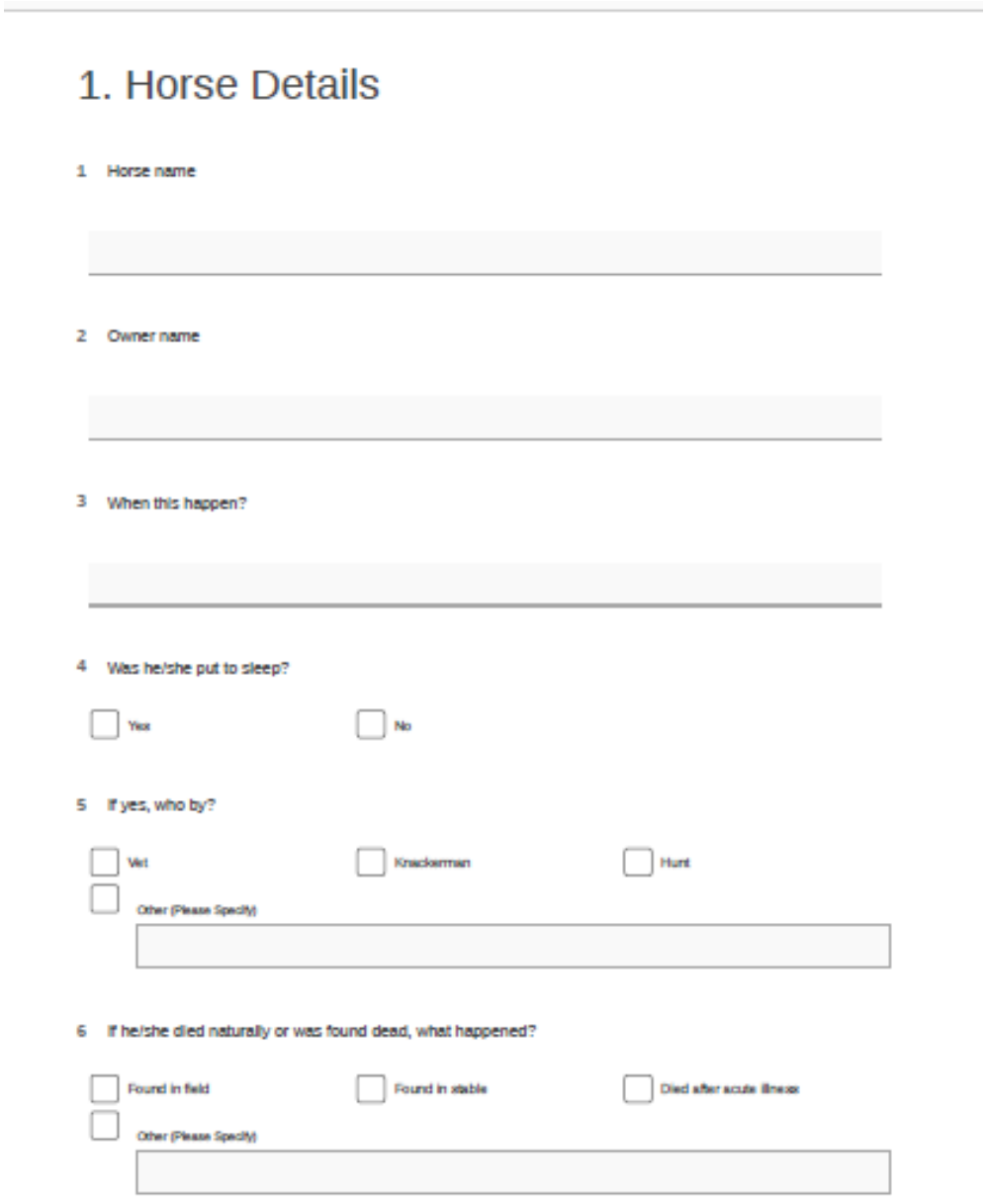
- [ ] Yes
- [ ] No

51 Has treatment for the condition(s) been completed?

- [ ] Yes
- [ ] No
- [ ] Ongoing
- [ ]

Other (Please Specify)

Appendix 3 – Mortality questionnaire



1. Horse Details

1 Horse name

2 Owner name

3 When this happen?

4 Was he/she put to sleep?

☐ Yes ☐ No

5 If yes, who by?

☐ Vet ☐ Knackerman ☐ Hunt

☐ Other (Please Specify)

6 If he/she died naturally or was found dead, what happened?

☐ Found in field ☐ Found in stable ☐ Died after acute illness

☐ Other (Please Specify)

7 If he/she was put to sleep, what happened?

- [ ] Colic
- [ ] Acute illness
- [ ] Chronic illness
- [ ] Osteoarthritis
- [ ] Fracture
- [ ] Laminitis
- [ ] Chronic infection
- [ ] Other lameness
- [ ] Other (Please Specify)

8 How did you reach the decision to have him her put to sleep?

- [ ] Severe/uncontrollable pain
- [ ] Hopeless prognosis
- [ ] Vet advice
- [ ] Long term problem
- [ ] Cost of further treatment
- [ ] Poor quality of life
- [ ] Other (Please Specify)

9 Did he/she receive any veterinary treatment for the problem/illness?

- [ ] Yes
- [ ] No

If yes, please specify

10 Other than PPID did your horse have any other health problems at the time?

- [ ] Yes
- [ ] No

If yes, please specify

11 Did the fact your horse had PPID influence your decision to have him/her put to sleep?

- [ ] Yes
- [ ] No

If yes, please give further details

12 Any other comments

Chapter 7

Factors influencing owner decision making regarding the management and treatment of pituitary pars intermedia dysfunction (PPID)

Factors influencing owner decision making regarding the management and treatment of pituitary pars intermedia dysfunction (PPID)

Summary

Pituitary pars intermedia dysfunction (PPID) is a chronic progressive disease that requires long-term treatment, predominantly administered by owners in their horse's home environment. Pergolide has been shown to be effective at reducing clinical signs of PPID, however, studies have demonstrated that some owners choose not to follow veterinary recommended treatment protocols. The owner decision making process around how to treat their horse is complex and influenced by a multitude of factors. This study aimed to understanding the context in which owners think about PPID and make decisions in relation to the treatment and management of their horse. Semi-structured individual in-depth interviews were conducted with 10 purposely selected owners whose horses had be diagnosed with PPID by the University of Liverpool Leahurst Equine Practice. Data were analysed using thematic analysis to identify codes and themes within interview transcripts. At total of six overarching themes were identified; disease tangibility, balancing management and treatment complexities, owners being experts in their own horse, having a horse centred approach, the vet-owner relationship and how health and happiness go 'hand in hand'. The themes demonstrated how treatment and management decisions were influenced by the impact of PPID on the horse's daily life and the visible changes observed by the owner. This perception was then built on and framed by the owners understanding of the disease, their in-depth knowledge of what is normal for their own horse and the nature of the vet-owner relationship. Decisions were driven by what owners' thought was best for their horse and enabling them to have the best QoL possible. However, this could be confounded by conflicting needs. The findings described here demonstrate how owner treatment and management decisions are influenced by a complex mixture of contextual factors and key drivers, providing a depth of understanding of owner's experiences, beliefs and perceptions that is not available through quantitative surveys.

Introduction

Long-term adherence to treatment protocols has been reported to be problematical in both medical and veterinary practice. Despite demonstration of treatment efficiency, poor treatment compliance has been reported in various chronic diseases such as osteoporosis

(Huas, 2010), asthma (Dinwiddie and Müller, 2002) and type 2 diabetes (Cramer et al., 2008). Compliance with recommended veterinary treatments such as dental procedures and surgery among companion animal owners has also been reported to be as low 30% (Kanji et al., 2012). Treatment choices in owners of small animals have been studied and are reportedly influenced by the vet-owner relationship and owner-pet relationship (Adams and Frankel, 2007; Lue et al., 2008; Kanji et al., 2012), as well as wider contextual factors such as perceptions of risk and trust (Maille and Hoffmann, 2013). However, understanding of the factors that influence treatment and management decisions among horse owners is limited.

Pituitary pars intermedia dysfunction (PPID) is a chronic progressive disease, commonly diagnosed in older horses (van der Kolk, 1997; McFarlane and Cribb, 2005; McFarlane et al., 2005b; McGowan et al., 2013a), requiring long-term management (Chapter 4). Pergolide has been shown to be effective at resolving clinical signs of PPID, but is reliant on regular administration by owners, in their horse's home environment (Chapter 4; McFarlane et al., 2017). There is evidence of poor owner compliance with veterinary prescribed treatment regimens for PPID (Hague et al., 2021). This is supported by the findings described in Chapters 5 and 6, which demonstrate that some horses may not be receiving veterinary recommended treatment, and that a small proportion of owners change or stop medical treatment without veterinary input. Pergolide administration has been associated with increased odds of survival (Horn et al., 2019), therefore unregulated changes to treatment may lead to increases in mortality and morbidity impacting on equine welfare.

Understanding the context in which owners think about PPID and make decisions in relation to the treatment and management of their horse may assist veterinary surgeons in communicating with owners and supporting them to make the best decisions for equine welfare. This can be achieved using a qualitative approach in contrast to questionnaire-based studies (Chapters 5 and 6). There is a growing awareness of the need for qualitative sociological studies in equine veterinary research, to investigate human-horse interactions and the impact they have on management decisions. The importance of human influence on horse care and the effect this has on equine health and welfare are increasingly being recognised. A range of human aspects have been investigated using surveys, providing useful insights into the prioritisation of health and quality of life (QoL) concerns among owners (Mellor et al., 2001; McGowan et al., 2010b; Ireland et al., 2011c), industry stakeholder attitudes towards and perceptions of equine welfare (Collins et al., 2010) and

factors influencing the uptake of biosecurity practices (Schemann et al., 2012, 2011, 2013; Crew, 2019). However, these questionnaire-based studies do not provide the depth of understanding around experiences, beliefs and perceptions which underpin how attitudes are formed or decisions made. Therefore, a variety of qualitative methods investigating the reasons behind owner and veterinary decision making for equine care are being slowing integrated into the veterinary literature. Thematic analysis and ethnographic studies have investigated management and training of race horses (Cassidy, 2002; Butler et al., 2019), narrative analysis was used to describe care of horses on livery yards (Birke et al., 2010), drivers influencing antibiotic prescription by veterinary surgeons where identified using thematic analysis (King et al., 2018) and grounded theory conceptualised owner perceptions of equine obesity (Furtado, 2019).

This study aimed to improve understanding of the factors influencing management and health care choices made as well as how owners perceive QoL in horses with PPID. The objectives were to investigate owner understanding of the wider issues and questions around PPID and its treatment as well as how their attitudes or beliefs may have formed; an in-depth exploration of relevant issues relating to treating and managing PPID for horse owners and how these affect decisions made around caring for their horse. The qualitative research methodology was used to portray and develop an understanding of the relative meaning of PPID for horse owners within different contexts, and describe the reasons for decisions regarding treatment and management.

Materials and Methods

Semi-structured interviews were conducted with owners/carers of horses with PPID to elicit perceptions of their horse's QoL and motivators for the management and health care choices made. Thematic analysis (Braun and Clarke, 2012) was used explore immediate and wider contextual influences shaping owner perceptions, decisions and behaviours. This qualitative approach was part of a mixed-methods study.

Sample

Owners were initially invited to complete a quantitative questionnaire the full details of which are described in Chapter 5. In brief, horse owners were recruited via the University of Liverpool Equine Practice (UoLEP) database. Using key word searches, owners of horses

diagnosed with PPID, based on elevated basal plasma adrenocorticotrophic hormone (ACTH) concentration, were identified and clinical records were reviewed to confirm that cases met study inclusion criteria. Information provided in the initial questionnaire was used to purposively select horse owners based on characteristics such as current PPID treatment used, premises type, PPID comorbidities reported and length of ownership. This ensured diverse experiences and a range of viewpoints. Owners were recruited to participate in the interview process by telephone where their PPID experience was confirmed and the interview process was explained. This was followed by a confirmation email or letter containing further details on the aims of the study and a consent form explaining confidentiality, anonymity and the right to withdraw (Appendix 1).

Data collection

Semi-structured one-to-one interviews were conducted using an interview topic guide (Appendix 2) that was developed and piloted with two fellow researchers and two horse owners. The topic guide used open questions to encourage meaningful discussion and was used to direct conversation between the researcher and interviewee. Each of the topics were included in all interviews and questions were carefully phased to encourage interviewees to express their views and recount their experiences. The focus was on gathering the information needed, however, the process was flexible and avenues of conversation were pursued for additional information on the interviewee's thoughts or opinions as appropriate.

Interviews were conducted at a time and place convenient for the interviewee. The majority were held at the premises where the horse was kept, with two taking place in a private consultation room at the UoL Equine Hospital. Interviews were recorded using a digital recording dictaphone and lasted between 43 and 80 minutes. For consistency, all interviews were conducted by the doctoral student, who is a knowledgeable horse owner with experience and knowledge of PPID, but not a veterinary surgeon. This meant owners were able to relate and open up to the interviewer without feeling they might give a 'wrong' answer. Not being a veterinary professional also meant the interviewer was likely to be less biased about the treatment decisions made by owners. However, the interviewer was associated with the UoLEP and this may have influenced any perspectives given by owners regarding vet-owner relationships.

Profiles of interviewees

A total of 10 owners were recruited to participate in interviews with a variety of PPID experience. Based on their questionnaire responses, interviewees had cared for their horses for a median of 16 years (range 6 – 25 years), and their horses were diagnosed with PPID between 2 months and 4 years prior to interview. Four interviewees cared for a single horse, one interviewee cared for two horses, one of which had PPID, and another cared for two horses both of which had PPID. Four interviewees were responsible for caring for multiple horses (≥2), at least one of which had PPID. Baseline questionnaire data (Chapter 5), showed that seven interviewees currently administered pergolide treatment, two of which had also used alternative treatments. One interviewee had recently stopped administering pergolide treatment and another had stopped using pergolide for a period and then restarted treatment. One further interviewee had never used pergolide treatment and instead used an alternative treatment. Interviewees reported a variety of clinical signs of PPID in their horses, with three having observed the overgrown curly coat associated with advanced PPID, while four reported recurrent episodes of laminitis and seven also reported that their horse had concurrent conditions such as arthritis. All interviewees were female and amateur 'hobbyist' horse owners, however, two were also livery yard managers and one ran a horse sanctuary. Horses were kept at a variety of premises including livery yards (n=4), rented premises (n=1), family farm (n=1), private premises (n=1), a horse sanctuary (n=1) and the owner's own livery yard (n=1). Quotes from each interviewee are presented with their individual identification code (e.g. P1).

Data analysis

Interview recordings were transcribed verbatim by the doctoral student and interviewees were allocated an identification code to ensure anonymity. Thematic analysis was undertaken following the iterative step-by-step approach described by Braun and Clarke (2006, 2012):

- Familiarising yourself with the data
- Generating initial codes
- Searching for themes
- Reviewing potential themes
- Defining and naming themes

- Producing the report

This approach is not specific to a theoretic framework and offers a flexible approach to examining issues and answering research questions. Thematic analysis offers an accessible form of analysis useful for examining different perspectives of research participants, highlighting similarities and differences and summarising key insights from the data (Cassell and Symon, 2004; Nowell et al., 2017).

Familiarisation

In this study the familiarisation process began during the transcription phase with initial observational notes being recorded throughout the process. Each interview was then actively re-read taking the time to think analytically about how the interviewee's experiences influenced their decisions and what was revealed by their accounts. Further notes were taken at this stage highlighting data potentially relevant to the objectives and research question.

Coding

The data were systematically analysed using codes to identify diversity and patterns in the data. The purpose of this study was to 'give a voice' to all owners' experiences of treating and caring for their horse with PPID. Therefore, a predominantly inductive approach to coding data was used. This meant codes were derived from, and closely reflected the content of the data. However, there was also a deductive 'top down' element to data interpretation as analysis was approached with specific research questions in mind to allow the aim of the project to be addressed. Everything potentially relevant to the research question was coded using a mixture of line-by-line and section coding depending on the richness of the content.

Coding of the first three transcripts was conducted by hand on printed paper copies of the transcripts. The initial codes generated were used as a framework to build upon when coding subsequent transcripts. The remaining seven transcripts were imported into commercial qualitative data analysis software (NVivo version 12) and coded using its node function. The first three transcripts were then revisited in NVivo, as codes had evolved, developed or combined throughout the process and new codes had been identified. Initial codes were mainly descriptive and became more interpretive as the process progressed by

interpreting collective meanings and experiences. This enabled analysis to go beyond simple description of the data by taking a deliberative, reflective and thorough approach. For example, an initial code was 'horses in discomfort can still be happy', which became 'setting acceptable QoL level' as it became clear that owners had set thresholds for how much discomfort their horse could experience before it started to affect their 'happiness' and therefore QoL. Another example was the code 'importance of field turnout', which became 'freedom to live as naturally as possible' to encompass the negativity owners felt about confinement to a stable, but that they also understood it was often a necessary part of management. Codes were reviewed a final time along with initial interview and coding notes, to ensure they appeared across more than one interview transcript and captured the salient features of the data.

Searching for, reviewing and naming themes

A theme "captures something important about the data in relation to the research question, and represents some level of patterned response or meaning within the data set" (Braun and Clarke, 2006, p. 82). Themes were actively searched for by reviewing the coded data and clustering similar codes together to identify unifying features and key differences. Codes were combined to form overarching themes that brought together and underpinned the data. Sub-themes were used initially to maintain context where several patterns within the data contributed to the theme in different ways. The relationships between themes were also explored to ensure they represented the story told by the data. To aid the process, codes were mapped onto an A0 pin board (Appendix 3). This was a fluid and iterative process, which involved grouping, reviewing and then re-grouping codes. The raw interview data and previous notes were referred to, in order to help guide which codes should be clustered to form themes and which themes were linked. This process also helped to add a level of analytical interpretation to the themes produced. To ensure the themes generated were representative of the data set and had sufficient data to support them the transcripts were re-visited and themes were checked against data extracts. At this stage some themes were re-phased to ensure they meaningfully captured all the relevant data.

Results

Extracted themes are presented in Table 1. Treatment and management decisions were influenced by the impact of PPID on the horse's daily life and the visible changes observed

by the owner. This perception was then built on and framed by the owners' understanding of the disease, their in-depth knowledge of what is normal for their own horse and the vet-owner relationship. Decisions were driven by what owners' thought was best for their horse and enabling them to have the best QoL possible. Owners interpreted QoL on an almost daily basis and based it on a balance of signs of happiness and health. Contextual factors influenced the decisions made around achieving what was best for their horse. Figure 1 outlines the overall goals that drove owner decision making and the contextual factors that influenced how these goals were achieved. Owner goals were distinct from other influencing factors as they represented the owner's overarching ambitions, while contextual factors impacted or shaped the owner goals. The goal of owners was to ensure their horse was both happy and healthy, and that their daily life was, at least in some way, influenced by what the horse itself wanted (as perceived by the owner). However, various factors were taken into account when reaching these goals, such as the owner's relationship with their vet, the impact of PPID on their horse's daily life and complexities around treatment and comorbidities. This meant owner goals and contextual factors sat separately but were intrinsically linked as shown in Figure 1.

Table 1: Themes representing influencers on health care choices for owners of horses with PPID and their perceptions of quality of life with representative interviewee quotations.

Theme	**Representative quote(s)**
Disease tangibility	*'I don't know what you are supposed to see with it, because she has never looked any different to me [ok] and she has not got the waviness of her hair or anything like that, none of that is showing which is usually a visible sign'. P10* *'I know he has got his Cushing's and his feet but he's not an ill horse, you know what I mean, he's poorly but he is not ill …truthfully I don't really think it (PPID) does that much, because he can still go out on the grass and he's fine'. P9* *'I don't feel massively that the cushing's has a huge effect on him sort of outwardly, he's not had the laminitis, he's not sort of struggled in that respect with'. P1*

Balancing management and treatment complexities	*'Well one thing I was worried about was keeping him in more than I was erm mainly because I knew you had to keep him stabled because of his weight but because the vet, the first test happened because he had, he was developing arthritis in his back end because he's had a serious injury 7 years ago…so it was a catch 22 do I put him out so he is walking around constantly or do I keep him in'. P2.* *'We know how she reacts to the Prascend, if its increased erm I don't know quite, if she did need to have it increased I don't know quite how we'd go about doing it without her going into zombie mode'…P7.* *'So I started him off on half a tablet and he had diarrhoea, and I thought well you know, you do with a paracetamol sometimes or whatever, with bute and stuff, so a few day and he got the runs erm, if I gave him a full tablet, because she said try and up it to a full tablet, you can up and down it, full tablet definitely diarrhoea, so it was like ok I'll just leave him on half for a while and he was half for about a week and a half, something like that, and he was still getting the runs with it and stuff and I thought that's got to be uncomfortable for him'. P5* *'He cannot control his temperature what so ever erm so obviously this summer's been really hard form him erm you know trying to keep the flies off him because if he gets a fly bite it's very, it's just a horrific reaction, and then on the other hand trying to keep him cool'. P4.*
Experts in their own horse	*'I can judge, I have had him for 17 years so I can judge if it's the same or not…you just know that he's enjoying it and I can tell when he is miserable'. P2*

	'It's always one leg and he will hold it up and that's how he tells me, you know you just know don't you [yeah] you get to know your own horse'. P9 *'I think you can tell erm just by being so close to them, if you were on full livery you probably wouldn't notice but because I'm here twice a day and I know him so well cos I've had him since he was 5 months old [yeah] you know I just know'. P4* *'It's all the normal things that she does, so I think you just know don't you, I think if you know your horse well enough you just know what she's like'. P7*
Horse centred approach	*'She hates being in, she is an outdoor horse she likes being out but she will stay in if the others stay in but she won't stay in a long time, well I don't let her because it is not natural… being able to go out, she loves it in that little paddock believe it or not, she is used to it, she loves being with the horses'. P10.* *'Monitoring her hay, she is a funny old so and so, she'll eat soaked hay for a few days and then she just won't eat it at all, so sometimes I might do 2 or 3 days and if she keeps eating it we just keep going and then if she stops I give her a couple of nets of dry hay'. P7* *'I mean when she wants to come in if it's a horrible day stand she's going I know you are up there are you coming to get me, she has a watch on, and other days she will go no get lost I want to stay out a bit longer so she will tell me when she wants something'. P6*
The vet-owner relationship	*'I think the majority of people should just go with what their vet has advised [yeah], I wouldn't take it any other way erm you know they are the ones who have done the research and are out in practice, I may know my horse but that's why we are a team so, and that's the way that people should look at it, I think,*

	you are only trying to do the best for my horse the same as I am so yeah'. P4 *'I know people come a long and say you need to try Laminase and you want to do this, no, I stick with what the vet said erm, and that's what I like and want to do'. P9* *'I think if the vets were a little bit more open to stuff and not so narrow minded and this is how much it costs, and it costs an absolute fortune and this and that, it puts a lot of people off'. P5*
Health and happiness 'hand in hand'	*'If she had bad health then she wouldn't be happy would she, you know if she was in pain or not feeling herself then she wouldn't be happy so I think they go hand in hand really'. P8* *'If you have got good health you are going to be happier, if you have got poor health you are bit down or whatever, so I don't know if we can have one more important than the other to be fair, not that we get it right all the time but we try anyway yeah… she has got to have her health to be happy if you know what I mean, if you don't have one you don't have the other'. P6* *'You know if we had a really bad illness and it was very bad then we would get unhappy after a while, and it would bring up down wouldn't it and whatever, so if your healthy you have got every opportunity to be happy'. P5* *'I would say, his happiness yeah because he has got to be happy but he needs to be in good health, if he was in really really bad health and unhappy then that is not good, but if he is in quite good health but happy I think that is ok as long as it is managed'. P9*

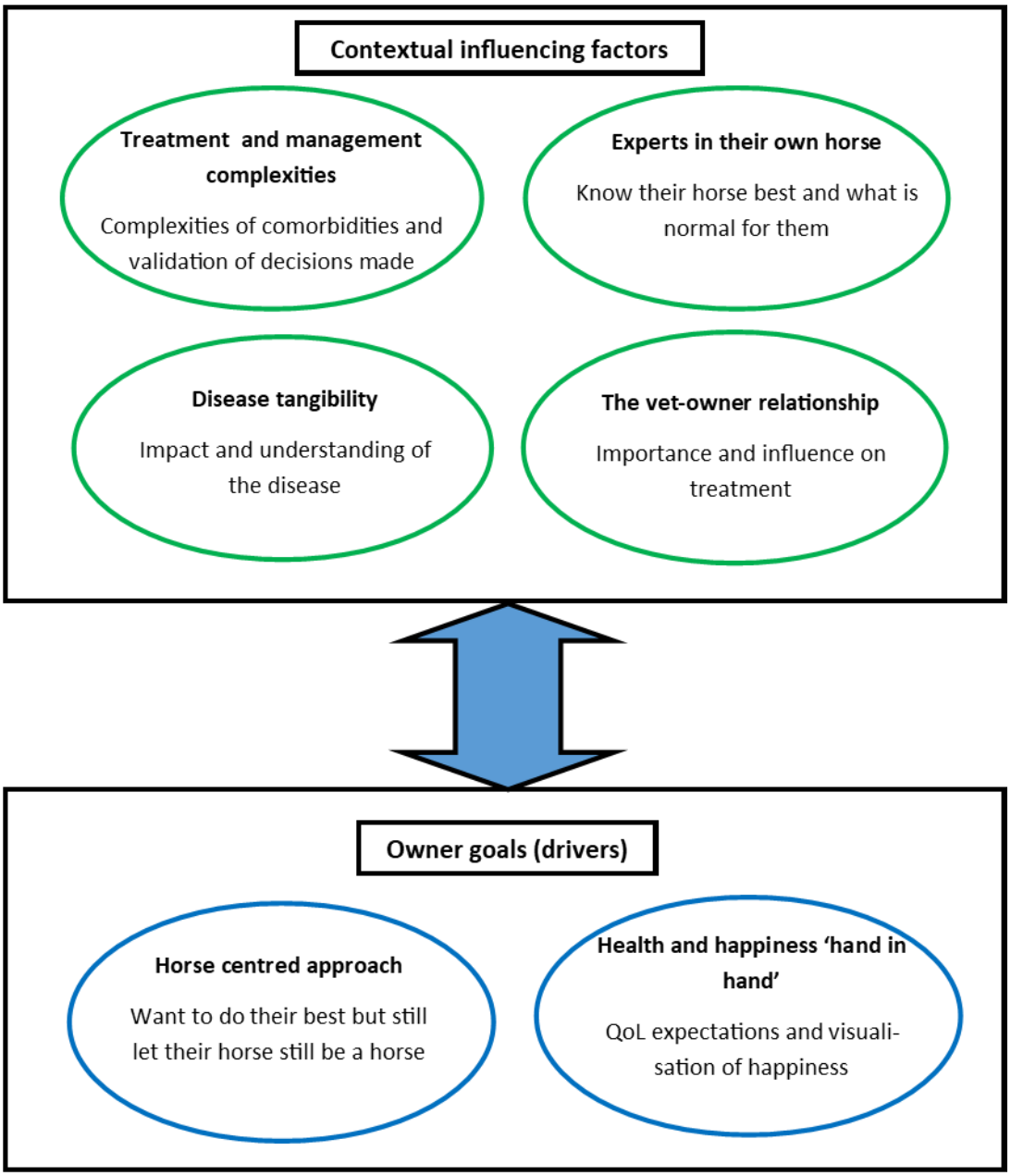


Figure 1: Contextual factors and owner goals influencing owner decisions around the treatment of PPID.

Disease tangibility

All owners had a daily ritual which they followed when caring for their horse, and how much PPID impacted on this influenced how they viewed the disease. If the disease was not perceived to be having a significant effect on their horse's day to day life or if their horse was not exhibiting what were seen as the 'typical signs', PPID became something abstract

and difficult to comprehend. Many owners described their difficulty quantifying the disease as they did not see their horse as 'ill'.

…'When he was first diagnosed he wasn't a typical Cushing's horse, he didn't have any of fur you know curly fur, he was just a normal pony, he was a healthy fit horse and he still is, I know he has got his Cushing's and his feet but he's not an ill horse, you know what I mean, he's poorly but he is not ill'…P9.

…'I don't know what you are supposed to see with it, because she has never looked any different to me [ok] and she has not got the waviness of her hair or anything like that, none of that is showing which is usually a visible sign, it is the eyes, something to do with under the eyes or, that's to do with laminitis isn't it'…P10.

When it came to understanding the aetiology of PPID most owners described how they found it difficult to comprehend, despite lots of information being available.

*…'but it's in veterinary speak, the deeper you get into it and want to understand more about it there's a point at which even I couldn't understand what they were talking about and I still don't understand about the ACP the A whatever's *laughs*'…P2.*

Some owners were also aware how a lack of understanding could have a negative impact on the horse's care.

…'I think that you could easily get lost if you weren't careful and I think that's a shame because it's not the human that suffers it could be the horse'…P4.

Owners of horses that had concurrent health issues such as equine metabolic syndrome (EMS) described how they found it particularly difficult to differentiate between diseases and apply this to what they observed in their horse. This lack of understanding added to the sense that PPID is an abstract concept that is difficult to define.

…'you know to try and make sure that the insulin, no I may be getting the 2 things but the hormones and everything like that, I find it very difficult to, to, I find it personally, you probably don't but I find it very difficult to differentiate between what is causing what, so the EMS and the Cushing's I don't know which ones causing which'…P4.

Disease tangibility was also influenced by whether they perceived their horse to have clinical signs of PPID. Whether observed in their own horse or not, coat abnormalities were by far the most talked about and recognisable sign of PPID. Some owners found it difficult to understand the diagnosis if their horse was not exhibiting this typical sign, while others referred to the fact their horse had a 'hairy coat but that was it'. Even when owners observed 'evidence' of PPID, if it was not perceived to have an impact on daily life, the disease remained indefinable. Most owners also took their horse's age into account when talking about PPID with many using the phrase *'good for his/her age'.* Owners did not expect their horse to have the same standard of health or to look the same as a younger horse.

…'difference between erm, apart from to look at tiffany you can tell with her coat she gets that ridiculous coat erm, but other than that to look at her, you know, she doesn't sink in round her eyes, her belly is nice and where, considering she's an old horse as well'…P3.

…'I am looking out for stuff more but not just because of the Cushing's because of his age as well, because you do keep an eye on them a bit more, with general things in themselves'…P5.

This different expectation for older horses was an influential factor in the decisions made around treatment, as owners described how they made different treatment decisions compared to those they would for a younger horse. Most owners were happy to use analgesics, such as phenylbutazone ('bute'), more readily and undertake less investigative procedures.

…'the quality of life for him is important now at his age so you know if I had to give him more bute I'd give him more bute even though it perhaps wouldn't be the best thing necessarily'… sometimes I think I'd quite like to have his erm, blood count done on him, in the sense of his kidney function and his liver just to see where he's at but, and if he was 10 year's younger I probably would but I think at this stage being 31 I just, you know, to a certain extent I kind of want to leave him in peace because he's just so old erm, so for me it's keeping him comfortable and plenty of food that's important when they're old boys'…P1.

Laminitis was also a major influencer on how owners perceived PPID. All owners, irrespective of their experience of laminitis, talked about an increased risk of laminitis associated with PPID and the impact it could have.

…'I think it's the laminitis more than anything that's the worry isn't is with them'…P1.

Owners find PPID difficult to fully comprehend and therefore do not always know what to expect, their perspective is also often influenced by a different expectation of health for older horses. While ACTH levels offer some clarity, this is not always enough for owners to base treatment decisions on.

Balancing management and treatment complexities

When making decisions regarding management and treatment most owners were influenced by conflicting needs and concerns. They weighed up multiple factors and then made decisions they felt were best suited to their horse's situation. These conflicting concerns usually included other age-related conditions which required different management solutions to PPID.

…'well one thing I was worried about was keeping him in more than I was erm mainly because I knew you had to keep him stabled because of his weight but because the vet, the first test happened because he had, he was developing arthritis in his back end because he's had a serious injury 7 years ago…so it was a catch 22 do I put him out so he is walking around constantly or do I keep him in'…P2.

This shows how owners considered and deliberated because different ailments require different approaches and how there may not necessarily be a correct answer. This tactic was also undertaken by some owners when it came to treatment. Ensuring correct treatment had to be balanced with factors such as side effects and cost.

…'I would possibly get it done in the autumn, which is what we decided, just to see, because we haven't got err to see whether it's gone up or whether it's come down or whether it's just stayed the same [yep], we know how she reacts to the Prascend if its increased erm I don't know quite, if she did need to have it increased I don't know quite how we'd go about doing it without her going into zombie mode'…P7.

…'I can't afford, you know, once the insurance run's out, it's another pound a day'…P2

However, cost was not a major influence in the majority of cases.

…'erm so yeah it has to come in to in but it's one of those, I just see it as you've got to eat, they've got to have the tablets, it's the same thing, it's just a cost that I have to have, it's part of horses isn't it'…P3

Treatment decisions were often influenced by perceived effectiveness of the treatment and severity of disease. One owner who currently used an alternative herbal treatment described how she would need to adapt if the clinical signs of PPID deteriorated.

…'but if I carry on giving him the chaste berry's and he goes downhill quite quickly they are obviously not doing the job their not preventing it enough, they're not keeping that at bay so then I'd probably try getting him on the tablets something that is a bit more hard hitting, if his disease whatever he's got is hard hitting him I need to fight back with hard hitting and if they're not enough then it will have to drugs to keep it at bay'…P5.

Many owners viewed ACTH levels as a valuable and definitive measure of their horse's health on which to base treatment decisions both initially and throughout monitoring.

…'she seems to be doing alright but as I say we will get her blood tested again in the autumn and see what that does and then we will go by what results we have got there and see whether we need to alter or change fiddle around with it or do something if we need to, and if we don't, if it still reasonably low we will leave it where it is'… P7

However, occasionally when ACTH levels did not respond as expected, or did not seem to fit with what the owner was observing in terms of health, this led to confusion and resistance. Owners needed to weigh up what they observed in 'the horse in front of them' with the 'science'.

…' this time last year they were 1100 and something after 6 weeks of me trying all this herbal stuff and all the stuff that they recommend that are the alternatives to prascend [yeah] it didn't work so then he went on the prascend so it went down to a really reasonable level 21 or something [ok] then in the spring he had it done again and it was still really low

erm so then you come to this autumn and its gone back up to 150 or 170 but after speak with the vet erm cause its cost as well, he's 20 years old how much of this would of happened anyway, erm … but it's just something to keep an eye on, so it has gone back up to 115 or so [ok] erm at the moment, but he's never, he's looking fine'…P2

A specific objective change was not always required for owners to perceive a treatment as effective, many owners considered the bigger picture of their horse's life overall when weighing up the difference before and after treatment.

…'It enables me to … erm I think for him to have more of a normal life as a horse'…

In terms of management decisions, laminitis was one of the main concerns and considerations. The majority of owners explained how concerns around laminitis meant they restricted their horses grazing and constantly monitored for any changes, both in their horse and the grass.

…'yeah he's actually been, he goes up and down like anything, I have to totally monitor him all the time because if he goes out on the grass he can only go out on crappy grass because if he goes out on anything good he will just come down with it [Laminitis]'…P9.

…'yeah because I am really aware of laminitis you know so, yeah, and I'd be careful you know with like spring grass and things like that, more than I would before previously'…P8

Some owners described how their horse had already been on a strict management routine for other issues, such as weight management, prior to diagnosis with PPID. Therefore, they had not felt needed to make major changes post diagnosis. Subsequently they perceived PPID as 'just something else to deal with' which had minimal impact on their routine or their horse's life.

However, despite their experiences all owners stressed the importance of constantly monitoring everything about their horse's behaviour and routine. This enabled owners to feel 'on top things' and in control of their horse's health.

…'I do keep an eye on him more erm, with his, just to make sure there's nothing going on with him as in he's looking depressed or looking unhappy or erm if he isn't sleeping because I can tell when he's sleeping obviously in his bed'…P5.

Multiple and sometimes complex considerations had to be undertaken for owners to achieve the overall aim of doing the best for their horse.

Experts on their horse

Owners put a huge amount of value on their own experience: this often stemmed from the fact they had cared for their horse for a significant proportion of the horse's life and therefore 'knew their horse best'. Owners considered their horses to be individuals and the amount of time spent with them, both in years of ownership and daily routine, meant they knew what was normal for their horse in terms of their personality and behaviour traits, allowing them to recognise when changes in management are needed or identify changes in health.

…'I can judge, I have had him for 17 years so I can judge if it's the same or not and if he's eaten every bit of it I know that I'm not giving him enough so I'll give him another couple of handfuls the next day'…P2.

Owners described both objective and subjective signs of health in their horses. Subjective signs included looking 'bright eyed and bushy tailed' or other behavioural traits unique to their horse, as well as how they interacted with their companions. Objective signs described included the texture of their coat, their body condition and appetite.

…'he's got a lovely coat [ok] yeah, he err, if his coat is a bit starry, if he's a bit tucked up err, he's really really very dramatic erm so you know when, you don't even, you can tell by his eye's erm yeah he's very easy to read [ok] he's so easy to read it untrue, so but his coat will be shinny erm his hair will she shinny and not breaking, you know his mane, erm his eyes will be bright erm yeah, but but when he's not well you know [ok], he's really really pathetic'…P4.

…'I don't think he's had any major difference with not being on it, from the eye or in his demeanour, he's stayed pretty consistent'…P4.

…'she's, you know bright eyed and bushy tailed'…P7.

Many owners described how their horse communicated with them to show that they were in pain or discomfort.

…'he just, he literally won't walk he will just limp on one leg, it's always one leg and he will hold it up and that's how he tells me, you know you just know don't you [yeah] you get to know your own horse and will just start limping and as soon as I open the stable he is in so, because he has had it since he was 9 and he's 15 now, so he's quite clever'…P9.

A deviation from the norm needed to be big enough to prompt the owner to question why the change occurred and therefore evoke a reaction. This appears to be key to owners recognising changing states of health. Owners described how they had the knowledge to be able to recognise what is normal for the animal, especially in terms of subjective behaviours. This was often the first thing owners noticed a change in.

…'just their mood and how they are looking in themselves, you know if Tiffany's very quiet and a bit subdued I would think oh that's not quite right, because she's normally like there bossing about or getting all the attention if someone comes or whatever'…P3.

Although the owner's familiarity with their horse sometimes meant more gradual changes could go unnoticed until they had a big enough impact to provoke action.

…'you don't realise do you how much it creeps up when you see them every day you're not aware of it until you actually stand back and start assessing things'…P7.

Many owners relied on past experiences and recounted stories referring back to past events to back up points they made. These past experiences, either positive or negative, were considered to be important and influenced management and treatment decisions currently being made.

…'I'd had a little pony before him Dillan who had really bad laminitis so I knew the signs anyway, but Dillan had got put to sleep, I wish I had known then what I know now because I wouldn't have let him get PTS erm but he was just in a bad way, and I've thought I am not doing it with you and I have just tried everything'…P9.

…'hopefully the natural way will help him because it's helped his arthritis and he's only on devils claw with that'…P5.

Another aspect which fed into the feeling that owners 'know their horse best' was the deep bond they felt with their horse. Most owners perceived their horse as a pet or members of the family. They owned their horse for pleasure and not to perform a job, meaning owners cared for them in the same way even if they could no longer be ridden. One owner described the difference between herself who owned horses for please and people who own their horse to compete.

…'because you look at competition horses they don't have them at an old age once their no good they are gone, they are sold on, erm children with their pony's when they grow out of them they're gone… your meeting people who are in it for the longevity, like me, Roxy is going to be with me for life whether I can ride him or not it doesn't made a difference [yeah] to me, he's my, you know he is part of my family'…P5

The emotionally driven and long-term relationships formed meant ensuring the best QoL possible for their horse was extremely important and influenced the decisions made around treatment and management.

…'to me they're just like pets I treat them like a treat my daughter to be honest yeah, they are like my babies…QoL is like massively important I would imagine for everyone, you know farmers with cattle and sheep and that might be a bit more kind of you know, but horsey people they're like pets aren't they so it is the QoL yeah really very important'…P3

Some owners described how they would make sacrifices to ensure their horse had the treatment they felt they needed.

…'for me it's important, their health is important and I've probably would not have a holiday if it meant having to treat my horse, you know what I mean, they are part of the family and it's important to keep them healthy'…P1

A complex mixture of emotional attachment, experience and knowledge of their horse underpinned how owners perceived their horse's health and therefore influenced the decisions made around treatment and management.

Horse centred approach

Owners all wanted to 'do their best' for their horse by providing an environment in which they were comfortable, happy and had as much freedom to 'be a horse' as possible. The method of management was horse led, using approaches that were based on owners giving their horse the ability to choose for themselves. All owners gave examples of how their horse communicated needs and preferences.

…'it could be absolutely hammering down in the summer time I'd think oh my god I've got to go and see if he's alright, so I'd go to the field and I'd give him a shout and sometimes he's come running to me and I'd open the gate and get him in the stable, so he was out the rain, and there were times when I'd go… and I'd see him and he's be stood with the herd all nice and snuggles up like that, no rug on or anything just all nice and snuggles up, and I'd go 'Rox are you coming in, come on' and he'd be like no, [laughter] and I'd say alright and I'd go home, and I'm alright with that now because he's chose he doesn't want to come in and he knows what I am offering him'…P5.

Owners also described reading the actions and behaviours of their horse to decide what management choices to make and when changes were needed. Their horse often dictated, to a certain degree, how much time they spent stabled/turned out and the type or quantity of food given.

…'she is a funny old so and so, she'll eat soaked hay for a few days and then she just won't eat it at all, so sometimes I might do 2 or 3 days and if she keeps eating it we just keep going and then if she stops I give her a couple of nets of dry hay'…P7.

…'now I gave her one [hay net] about 1pm which is half a kilo, probably about 2pm she will start, excuse me she will whinny at me, I want another hay net now, she will let me know'..P6.

Owners believed their horse was able to communicate with them through their behaviour and this helped to steer them towards making the right judgment regarding management depending on the behaviour displayed.

Freedom for their horse to have some form of turnout was perceived to be vitally important for happiness. Owners described how their horse needed time and freedom to exhibit the natural behaviours of a horse and interact with others, even if turnout or grazing was restricted. All owners talked about how their horse needed time to socialise and exercise.

…'so he can actually go out and have a bit of grass, ok he can't go out and have you know an acre or half an acre or a paddock you know he's got to be rigorously maintained, erm so he can have strip grazing, but he has, up until this point, been able to live a fairly normal EMS and Cushing's horse type lifestyle…I mean you know they're not really horses that can go out in acres of grass erm so they should be able to have some kind of turn out whether its limited like he has in a paddock that's probably about the size of this (barn/feed room) maybe then strip graze it a bit bigger, so he can keep on top of it erm, but outside, they need to be outside he needs to be able to go in the arena and he needs to be able to run and have a good buck, he needs to be able to roll, he needs to be able to do all the things that horses do'…P4.

….'she hates being in, she is an outdoor horse she likes being out but she will stay in if the others stay in but she won't stay in a long time, well I don't let her because it is not natural… being able to go out, she loves it in that little paddock believe it or not, she is used to it, she loves being with the horses'…P10.

Owners described a kind of controlled freedom where the restriction of grass intake was considered an important management factor. One owner in particular described how she had never let her horse have …`*full rein to eat himself to death'…P1*.

Although stabling their horse was part of the day to day management for most owners, confinement to a stable for longer than was considered normal had negative connotations.

…'it is just like being in a prison really isn't it just in a square box no matter how good the scenery is'…P6.

…'he is in a little pen like that where he can see all his friends he's not stabled stuck away, stabled stuck away is not a good QoL not being able to eat, not being able to run with his friends, that's not a good life that's not fair, I think at that point it's time to say enough is enough this is not a good QoL'…P9

Management practices were generally preventive with many owners describing how their horse had been on a strict management regime before being diagnosed with PPID, therefore they had not had to make any changes as a result.

…'I think his routine looks after him anyway [ok yeah] it would be different if he was left out more and left to his own devices'… P5

How owners interpreted their horse's behaviour was the main influencer in day to day decisions regarding management. Owners felt that this approach enabled them to determine what was best for their horse.

The vet-owner relationship

Most owners did basic research about PPID online or by reading articles, but mainly relied on their vet to provide them with information. Owners felt that vets played an important role in the care of their horse and were a great influence on the treatment decisions owners made.

…'I think I just googled cushings and you know then there were help site and things and I just read everything I could about it, as you do'…P8.

…'you know the vets they are up to date with most up to date information I just follow what they say, there would be nothing that I could suggest for mine anyway that you wouldn't already of suggested'…P3.

Owners respected and put their faith in their vet, looking to them as the primary source of advice. However, owners also felt that they were able to have an input in their horse's care and work in partnership with their vet towards a common goal. Owners described how they saw vets as experts in the disease but themselves as experts in their horse.

…'I think the majority of people should just go with what their vet has advised [yeah], I wouldn't take it any other way erm you know they are the ones who have done the research and are out in practice, I may know my horse but that's why we are a team so, and that's the way that people should look at it, I think, you are only trying to do the best for my horse the same as I am so yeah'...P4.

Most owners valued the advice of their vet over other sources such as peers.

…'I just stay with my vets to be honest with you, I do, I know people come a long and say you need to try Laminase and you want to do this, no, I stick with what the vet said erm, and that's what I like and want to do erm, and I have always stayed with my vet practice for 20 years and I am happy with them and I am happy with the way everything is and I am not just saying it because your here, I really do, I am happy'…P9.

However, there were factors which caused the vet-owner relationship to fluctuate. Some owners had feelings of resentment around the cost of treatment.

…'but then you think ok if more horses are healthy then vets would be out of a job and not earning the extortionate money that hospitals make'…P2.

…'so I think if the vets were a little bit more open to stuff and not so narrow minded and this is how much it costs, and it costs an absolute fortune and this and that, it puts a lot of people off, and that is not for the welfare of the horse, ultimately it is the horse that loses out'…P5.

Although for most owners cost was not the main consideration it was a concern, especially when dose increases were required, it then became a potential influential factor in the level of treatment received.

…'I'd be quite happy to put him on 1 a day I could, you know I really would because obviously it cost me, I was so thrilled when I had my ACTH levels come back at 2 and he was fine, because in the summer he wasn't it went up to 2 and a half so it's come back down, that for me that extra half a tablet, I know it's only 50p but when it's in the grand scheme of things it all adds up'…P4.

The vet-owner relationship is trust based and can be fragile: a single bad experience can have a long-term effect. One owner described how her experience of a misdiagnosis by a previous vet had affected her perception.

…'we got her on prascend but I think it was too late by then [I see] we tried to manage it but it got too bad, I think probably if we had caught it earlier, without me arguing with one

of the vets because I know my horse better, even though he's a vet, I know when my horse has laminitis and it was laminitis and I think possibly if it had been diagnosed earlier we wouldn't of had the rotation and whatever it is, I think if I saw him now I would punch him,'..P6

Owners liked to feel they were in control of the treatment and management decisions being made. Many owners described how the vet put decisions back in their hands, so although they were following veterinary advice, they were ones in control and making the final decision.

…'this is what needs to be done how you do it is up to you but we are here for support if you need anything give us a ring'…P9.

The vet-owner relationship plays an extremely important part in the decision making process of owners. Although owners are understandably heavily reliant on the advice from their vet, the relationship needs to be managed with care to enable owners to make the correct decisions, especially around treatment.

Health and happiness 'hand in hand'

To owners, the term QoL encompassed their horse being in good health both mentally and physically. Ensuring their horse had good physical health was a step towards ensuring good 'mental health' and happiness for their horse. Owners descried how happiness and health were intrinsically linked and that you could not have one without the other.

… 'she has got to have her health to be happy if you know what I mean, if you don't have one you don't have the other'…P6

… 'I think it's hand in glove, I don't think you can have one without the other, if she hasn't got her health she probably wouldn't be very happy'... P7

'if she had bad health then she wouldn't be happy would she, you know if she was in pain or not feeling herself then she wouldn't be happy so I think they go hand in hand really'…P8

When asked specifically about their perceptions of QoL, owners described key areas which underpinned how they constructed QoL. All owners expressed how freedom from pain and

the horse's ability to do normally daily activities, such as turnout and socialise, without impingement were the most important considerations for a good QoL.

'she's happy when she is not in pain and she can go out in the field and not have to limp that's her happiness I think'…P10

…'I said 7 years ago if my horse can't live in a field and run round with his friends then that's it, I will call it a day but he's doing that'…P2

…'Interviewer: what do you think makes a good quality of life for your horses?

Participant: I think they like their routine, they know their routine erm being with their mates and erm our stable are like an American barn type so they're not high partitions, so when they are in, their always in each other's space, you know, erm I think that's important for them, because they are all getting on now … errr, having their friends around them and having their routine and being pampered really I suppose, and keeping them, obviously keeping them healthy and pain free erm that's priority for me'…P3

Many owners visualised their horse's health through their demeanour, making it an important symbol of health. Ensuring their horse was happy and engaged was considered to represent good 'mental health' and was just as important as physical health for a good QoL.

…'She is perky, she is alert, she enjoys herself, so I think a stark coat or depression or things like that, I think would indicate … just need to check things'…P6

…'she was depressed definitely yeah, yeah and you know I couldn't have kept her like that because that it's not fair really, she just had no interest in life just you know head down not interested in anything, not reacting to anything, like a zombie really'…P8

…'their health is obviously very important but if they were suffering, if their mental health was suffering because of what I was doing to keep them physically well they couldn't have quality, a good quality of life'…P3

In some cases, discomfort was overlooked if owners deemed their horse's demeanour to be happy. Two owners in particular talked about how discomfort can be misinterpreted or

missed all together by owners. Highlighting how owners may misunderstand what their horse is communicating to them and how they might come to overlook physical signs of pain if their horse seems 'happy'.

…'it depends on the level of discomfort erm and I think you put low grade laminitis on the map, the reason that a lot of us probably miss it is because they are happy'…P4

*…'he can be happy and still have something wrong with him and then you'd measure up hang on how much is it hurting him, but if he's happy, I know when he snapped his suspensory he must have been in some sort of pain but he's eye's didn't tell me that he's really miserable'…*P2

Owners did recognise the need for 'short term pain for long term gain' with most owners describing how they would compromise happiness in the short-term if it meant a better QoL in the long-term. Owners generally set their own limit on what they felt was a reasonably amount of disruption to their horses QoL. Many owners put a time limit on disruption to QoL before it became unacceptable.

…'I've given him until err probably the end of March beginning of April, if he's not better and can't go out then I've made the decision already that I'm going to call it a day, because his QoL needs to be that he can go out, he's a horse that needs to go out'…P4.

…'I think if it's going to be a short term, you know, a couple of weeks erm obviously she had to come up here [Leahurst], she was up here a few times erm then for erm, it's good for her in the long run but if it was going to be like err you know she is going to be on this medication for the rest of her life and that's how it's going to make her, I'd have to consider her happiness because she's be suffering, her mental health would be suffering'…P3

Other owners put a limit on the amount of discomfort they would accept their horse being in. This could be in terms of the level of pain observed or how long the pain was observed for. Often what was seen as a small amount of discomfort was deemed acceptable in the longer term.

'if her stiffness goes in to her being very lame and very sore, or she get a really bad bout of laminitis then, no, I would have her put to sleep'…P7.

*…'he will struggle on here but once he gets on there (the field) he is like a rocket so, your like when do you call time you know'…*P9

Some owners anthropomorphised their horse's feelings and needs to make them more relatable and justify their decisions or actions for QoL management.

'I am thinking about my patients here, you just wouldn't do it would you, an old person stuck in the house on their own poorly, nobody to visit, nobody to see, there is no difference in a horse you know'…P9

… 'you know it's like old people isn't it you know they've usually got their aches and pains and stuff but they crack on and they do what they do and try and live their life as best as possible, and I think the horses do that obviously'…P3

Although happiness and health were both considered important for QoL, owners were more in tune with their horse's demeanour and 'mental health' than their physical health. When asked about the balance between health and happiness when it comes to QoL for their horse, owners more readily referred to their horse's happiness, frequently considering demeanour before aspects of physical health in their response, considering how their horse looked in terms of manner and interactions. Owners generally found these aspects easier to read than more physical or clinical issues.

…'just their mood and how they are looking in themselves, you know if tiffany's very quiet and a bit subdued I would think oh that's not quite right'…P3

…'you can tell by his eye's erm yeah he's very easy to read'…P4

*…'she's nosy, she is in your pockets, she is all over the place, it's not like she's you know how some of them get that depressed look, she is not like that, no she is not like that'…*P6

Owners perceived QoL to encapsulate both health and happiness. This was underpinned by key factors such as, their horse's ability to do daily activities, reading their horses demeanour and perceived level of discomfort. Owners used their expertise in their own horse to develop their own pre-defined idea of what happiness and health meant for their horse and to what extend they would allow it to be affected.

Discussion

This qualitative study provides insight into factors which influence owner decision making around the treatment and management of PPID as well as how QoL is perceived in the older horse. The overarching themes described here demonstrate how owner treatment and management decisions are influenced by a complex mixture of contextual factors and key drivers, providing a depth of understanding of owner's experiences, beliefs and perceptions that is not available through quantitative surveys. This study identified six themes which influenced and drove owner decision making around the treatment and management of their horses with PPID. Owners were driven by their want to do their best for their horse and ensure that they had a good QoL. Decisions to achieve these goals were influenced by their understanding of PPID and how it impacted their horse's daily routine, the communication received from their veterinary surgeon, their in-depth knowledge of their horse as an individual as well as other issues and conditions they needed to consider. The reasons behind human behaviours and decision making are well established within human health care and areas such as medical psychology. In human medicine, qualitative research plays a vital role in understanding the patient's behavioural response to disease. Qualitative insights have been used to describe and model areas such as; treatment and information seeking, attitudes and response to clinicians (Marshall et al., 2004; Walter et al., 2014). However, seeking to understand human behaviour in relation to the care of animals is still novel, especially in equine veterinary medicine.

Qualitative thematic analysis, as described by Braun and Clarke (Braun and Clarke, 2012), provided a structured analysis method for exploring the relevant issues rather than a specific approach to conducing qualitative research. This meant it provided an accessible and flexible approach to data analysis. Thematic analysis has previously been queried as an analysis technique because it was perceived to lack interpretive depth, however, this was because until recently, the method was poorly defined (Nowell et al., 2017). Braun and Clarke set out a detailed framework which provided a robust and systematic approach to coding interview transcripts (Braun and Clarke, 2014). The level of interpretation of the patterns identified is down to the researcher (Braun and Clarke, 2012). This study attempted to go beyond simple description of the data to add a depth of understanding to the decisions made by owners, however it was beyond the scope of the methodology used to establish a theoretical model.

This study recruited owners based on their experiences of PPID reported in the quantitative baseline questionnaire (results of which are described in Chapter 5). A diverse range of experiences and situations was achieved. However, recruitment was restricted to owners registered with UoLEP meaning geographic location was limited and veterinary involvement and experiences may have been similar. All interviewees were female amateur hobbyist horse owners, which is likely to reflect the main demographic of people who care for horses into their old age. The type of horse ownership may have influenced some areas such as their relationship with the horse as a companion or pet, therefore, the results presented here may not be entirely relatable to other owner situations, such as competition yards or riding schools. Although many of the themes are likely to cross over, owner goals or perceptions may differ as the owner-horse relationship includes a spectrum of opinions and approaches to horse care which can vary depending on how the horse is kept and for what purpose. For example, 'livery yard culture' has been described as an important influence on people's attitudes towards management and care (Birke et al., 2010) while amateurs and professionals have been reported to differ in their feeding practices (Harris, 1999) and decision making around treatment of conditions such as colic (Scantlebury et al., 2014). Competition and riding school horses have 'a job to do' therefore owner perceptions of QoL and priorities are likely to diverge from that of the leisure owner. Additionally, pergolide is not permitted under competition regulatory bodies. This means treatment decisions of competitive owners might significantly differ from leisure owners.

The number of interviews undertaken was pre-determined by the doctoral student and supervisory team. It was agreed that due to the demographics of the target population, ten interviews would be sufficient to reach a level of saturation in the data. All interviewees were leisure owners registered with the same veterinary practice and in a small geographical area, meaning there were likely to undertake similar management practices. Although this is a relatively small number of interviews there was some evidence of data saturation. During the iterative process of coding transcripts, codes were developed using the first three transcripts and then reviewed and re-visited once all transcripts were coded. During this process few new or additional codes were identified, suggesting a level of saturation had been reached despite the relatively small number of interviews. A larger sample incorporating multiple geographic areas and owner disciplines would require a larger sample and, as discussed above, may identify themes that differ from those described here.

All interviews were undertaken by the doctoral student, who is a knowledgeable horse owner with experience and knowledge of PPID, but not a veterinary surgeon. The intention was that owners would feel comfortable discussing treatment decisions openly. However, the project's veterinary supervisors, one of whom was practising at UoL Equine Practice, were named on the information sheet and consent form (as required for ethical approval). This association could potentially have influenced owner answers especially around the vet-owner relationship. However, this did not appear to be the case with owners openly discussing negative aspects of the vet-owner relationship, such as the cost of treatment and lack of options. Some owners did assume the interviewer had a certain level of knowledge or status, and this perception may have caused a level of social desirability bias.

PPID is a progressive disease with a wide variety of clinical signs (McGowan et al., 2013a). This made it difficult for owners to comprehend, especially when it was coupled with comorbidities that caused similar issues. Owners described their familiarity with the more advanced or obvious signs associated with PPID, such as a curly overgrown coat and laminitis. However, there was a clear lack of recognition of other more subtle or progressive clinical signs. This was coupled with the fact that owners expected their horse to look different as they got older and meant owners were likely to dismiss some signs as related to age rather than PPID. ACTH levels provide an objective measure for the disease, however, as discussed in Chapter 4, they do not always relate to clinical signs observed and pergolide treatment does not necessarily result in a return to within seasonally adjusted reference intervals (Rendle et al., 2013). This lack of correlation added another level of complexity, as owners were observing visible changes but no change in ACTH levels or vice versa. Owners have been shown to be effective at subjective evaluation of treatment efficacy (Gerber et al., 2011), however this is likely to be more challenging if owners are unsure what indictors to look for.

Disease (in)tangibility or complexities may be reasons owners looked at their horse's overall health when it came decisions around treatment. Medical studies into patient perceptions of new treatments have also shown the overall effect on QoL is as important as specific improvements in symptoms (Marshall et al., 2004). Equine studies have also demonstrated that evidence of efficacy is not a requirement for compliance (Goyen et al., 2017; Manyweathers et al., 2017), however this might be because owners have a different perception of what proves effectiveness for treatments. A study investigating owner decision

making around colic described how owners were influenced by contextual factors such as their previous experience, second hand experience, sources of information used and perception of the severity of the clinical signs demonstrated (Scantlebury et al., 2014). Lue et al, (2008) reported that one of the main reasons for owners not complying with veterinary care in companion animals is that treatment seems unnecessary. If owners are unable to understand PPID or accurately recognise its signs, then owners may be more likely to think treatment is unnecessary and therefore not to comply with treatment recommendations.

The lack of lay friendly information available to help owners understanding was also highlighted as affecting disease tangibility. This means owners are likely to use their own lay beliefs (experts in their own horse) to develop management and treatment strategies. The impact of lay beliefs on health behaviours, such as management of osteoarthritis (OA), have been investigated in human sociology (Morden et al., 2011). Patients put chronic conditions such as OA into context by describing how much it affects their life, for example whether it stops them doing normal daily activities such as working or hobbies (Donovan, 1991). Similarly, owners put PPID into context by assessing how much it impacted on their horse's normal daily routine, rationalising changes into their frame of reference and experience. In human medicine, findings have led to the recognition that clinicians need to build upon self-management approaches, supporting the existing beliefs and strategies of chronic illness sufferers (Kennedy et al., 2007). However, this is hindered when self-care is deemed outside of the clinician's perspective and boundaries are placed on patient participation. Veterinary surgeons need ensure they look at the wider context of the situation, not just clinical signs and ACTH levels, and fully engage with the aspects of care owners are already undertaking when setting out management and treatment plans (Rogers et al., 2005).

Older horses are more likely to have multiple comorbidities (Ireland et al., 2011b; Ireland et al., 2012b; Ireland, 2016; Welsh et al., 2016). This meant owners were often dealing with multiple conditions which needed consideration when it came to treatment and management approaches. Consequently, many owners were not considering PPID as a single entity, but weighing it up with various other conditions and other treatments. Faircloth et al. (2004) described a theory of 'biographical flow' where other comorbidities, such as diabetes, influenced the impact of a stroke of a person's life. Whether the stoke was major or minor, the part it played in the person's life was dependent on other illnesses. This is echoed in

how owners described the impact of PPID. Another study investigated non-adherence to Type 2 diabetes, and found that treatment complexity, such as number of medications being taken, route of administration and frequency of administration as well as additional directions, increased the likelihood of non-adherence (de Vries et al., 2014). This is likely to translate to owners who have more than one treatment or management decision to consider alongside pergolide administration. Another factor which added to the complexity of decision making was experience of side effects. It has been reported that concerns about, or experiences with, adverse events are the most common reason for the discontinuation of certain diabetes treatments (de Vries et al., 2014). Similarly, for owners who had experience of side effects this became an important consideration when starting pergolide treatment or changes to dose were recommended.

Among the many factors being considered by owners when it came to balancing management and treatment, perceived risk of laminitis was central. All owners talked about an increased risk of laminitis associated with PPID and this caused concern. Previous studies which have investigated motivators and barriers to undertaking preventive measures such as vaccination (Schemann et al., 2013; Goyen et al., 2017), identified perceived physical risk of illness as a key factor. Maille and Hoffmann, (2013) described how physical risk needs be high enough to induce fear, but if too high, may cause denial which inhibits its effect. In this study, laminitis did invoke a level of fear and owners described undertaking interventions if they thought the risk to their horse was high. Unlike other clinical signs of PPID, laminitis caused disruption to the normal daily routine in a similar way to that described by owners who had experienced colic (Scantlebury et al., 2014). Owners drew upon their own personal experience and knowledge of laminitis to inform changes in their management strategies which enabled them to 'keep on top of things' and constantly monitor their horses which reduced the fear factor. Nevertheless, it is still probable that owners who think their horse is at a higher risk of getting laminitis are more likely to comply with management and treatment recommendations. Some owners described how they had a strict management regime in place prior PPID diagnosis for managing other conditions, such as obesity or EMS, and therefore had not needed to make further adjustments to manage PPID. It has been described how stroke patients believed that changes to everyday life for management of diabetes, such as diet and exercise, may also help reduce the risk of stroke reoccurrence (Faircloth et al., 2004). Similarly, it is likely that some owners think their current management approach is sufficient to manage PPID in addition to concurrent conditions.

Owners felt that good management included aspects which implicitly reduced the risk of laminitis, their biggest PPID related concern. These aspects mainly related to controlled access to grass and were more active depending on individual experience of laminitis.

Owners felt they knew their horse best, putting huge value on their own experience, basing most of their decisions on their expert knowledge of their horse as an individual. Studies have described the emotional bond between owner and horse, with Keaveney (2008) describing it as 'unlike any other bond with companion animals', demonstrating the depth of connection felt, especially among amateur riders. This connection is likely to be magnified in owners of older horses who have cared for them from large proportion of the horse's lifetime. The same study described how owners were able to interpret their horse's facial expressions and gestures (Keaveney, 2008). If these subtle indicators deviate from what is considered normal for that individual horse, owners used that as an indication something is wrong (Scantlebury, 2012). This supports the findings of this study, where owners described how they are able to read their horse's body language and interpret behavioural indicators to assess their health and act accordingly. Studies have also highlighted that the way owners view their horse is an important influence on the way they manage and care for their horse (Hausberger et al., 2008). It was apparent that owners of horses with PPID had a very close bond with their horse, often describing them as part of the family, and this was reflected in how they cared for and managed their horse as an individual.

Turnout and access to grazing was a vitally important aspect of management. The main horse-centred focus was ensuring their horse had turnout as this was associated with positive natural behaviours, even when it sometimes conflicted with restricting grazing and added balancing management and treatment complexity. Butler et al., (2019) reported that 'keeping the horses' lives as natural as possible' by providing grazing was identified by stakeholders as an important factor in the QoL of racehorses. Studies investigating perceptions of equine behaviour have stressed the importance of natural grazing and socialising behaviours (Butler et al., 2019) and here owners wanted to try and recreate these natural behaviours as much as possible. As a result, stabling their horse for long periods was perceived negatively. This view may also be influenced by the link between stabling and negative behaviours such as weaving and crib biting (McGreevy et al., 1995a,b). When it came to daily decision making around routine, owners felt their horse was able to communicate with them regarding management decisions, in a similar way to how

they interpreted body language and behaviours to assess health. By letting their horse have input into their routine daily routine owners felt they were able to give their horse enough freedom to exhibit natural behaviours while maintaining overall control. Studies have reported that management practices change as horses age and that management and health care decline, especially if horses are retired (Ireland et al., 2011a, 2011b). However, this study shows that owners were putting careful consideration into the management of their aged horse.

The vet relationship was important, primarily as a source of advice and information but, could also be negative with some bad experiences relayed, demonstrating its fragility. The importance of the vet-owner relationship described here is likely to derive from a lack of lay information sources in the owner's social context, for example a lack of other owners in a similar situation on their livery yard. This meant owners turned to their veterinary surgeon more readily for advice. Consequently, veterinary surgeons became the main social influence, making the vet-owner relationship extremely influential. Social risk has also been shown to effect health behaviours (Hausenblas et al., 1997; Hausberger et al., 2008). Buckley et al. (2004) described how different owner groups access different information sources in different situations, demonstrating how complex information seeking behaviours are. Different social contexts, such as livery yard culture, have been shown to be influential in decision making (Birke et al., 2010; Scantlebury, 2012). This means lay sources of information are often reported to be the first point of reference for owners regarding their horse's health, rather than their veterinary surgeon. However, this kind of social context was not found to be a key influencer in this study. Studies in small animal medicine show that owners often have a strong relationship with their veterinary surgeon and that this makes them more likely to comply with recommendations (Brown, 2018). However, this relationship is dependent on the communication skills of individual veterinary surgeons, for example, how well they explain the reason for treatment recommendations and ability to educate the owner about the animal's needs. Owners who do not comply with treatment are likely to not have enough information to make the best decision (Lue et al., 2008). The strong influence of the vet-owner relationship means behaviour change strategies may be relatively simple to implement. Cost was an important consideration which could influence the vet-owner relationship as some owners felt resentment around the price of treatment. The upkeep of a horse has significant cost impactions such as livery, feed and insurance, though the amount will differ with different owner management systems. One owner stated

that pergolide treatment costs around £1 per day. Adding this to the cost of upkeep, likely for the rest of a horse's lifetime, means cost is a consideration in many cases. One study including 25 owners reported that many would be willing to pay up to $1000 per year (approximately £730) to medicate their horse with PPID (Schott et al., 2020b). Similarly, in this study many owners described how they would make sacrifices to ensure their horse got the correct treatment, highlighting the lengths owners would go to, to ensure their horses QoL.

QoL has been defined as encompassing feelings as well as physical health (Wojciechowska and Hewson, 2005). In this study, when owners were asked about their perceptions of health and happiness, they identified an inherent link between mental ('happiness') and physical health, describing how you cannot have one without the other. This underpinned the development of the theme 'health and happiness hand in hand'. In small animal medicine, QoL scores such as FETCH (Freeman et al., 2012), have been developed to assess and track QoL. This measures some of the indicator's owners described in the current study, such as eating and exercising. However, horse owners also relied on more subtle changes in demeanour to indicate QoL, which are difficult to account for or define in a questionnaire. QoL has previously been reported as an important consideration for owners of geriatric horses (Ireland, et al., 2011c). In this study, owners visualised what a good QoL meant for their horse and this was a key driver behind decisions and behaviours. This may influence treatment and management decisions in a similar way to how parents are involved in the decisions surrounding treatment in paediatric care. Parents are involved in the decision making process and are motivated by what they perceive as best for their child (Whitney et al., 2006; Brown et al., 2008). However, owners perceived the QoL of geriatric horses to be different to that of younger horses, with many suggesting that a low level of discomfort was acceptable, as long as it did not affect the horse's demeanour. Studies investigating the QoL of older patients describe how may perceive conditions such as OA as an accepted part of aging (Marshall et al., 2004). As owners anthropomorphised the feelings and actions of their horses, it is likely that this attitude seen in human patients is reflected in owners' attitudes towards the QoL of their horse. This may be one of the reasons why studies report an association between increasing age and a reduced QoL in horses (Ireland et al., 2011c), the same trend is also seen in dog owners, despite the fact that a strong emotional bond is also associated with an increased QoL (Marinelli et al., 2007). It has been reported that cat owners perceive QoL to be more important than quantity of life (Reynolds et al., 2010). This

also seems to be the case here: owners were confident they knew what a good QoL meant for their horse and would only let it be compromised to certain extent. Similar to other studies, the extent was dependent on expected QoL post-treatment (Ireland et al., 2011c) and the owner's individual experiences of the condition (Schemann et al., 2012; Scantlebury et al., 2014).

Conclusion

In summary, this study described owners' approaches to managing and treating PPID. Although limited to clients of a single veterinary practice, the themes identified here provide novel insight into owner decision making in relation to PPID. Owners were mainly driven by the want to do the best for their horse and ensure a good QoL, considering themselves to be experts in their own horse. However, this is often hindered by a lack of understanding of the disease and what indicators to look out for. Owners were influenced by comorbidities of geriatric horses as well as complexities in balancing management and treatment. The relationship with their veterinary surgeon was influential. To improve compliance with treatment and management recommendations, it is important that veterinary surgeons communicate clearly while considering the wider context of the owner's situation, including current management strategies, how they perceive QoL for their aged horse and other conditions they may be dealing with. Veterinary surgeons need to ensure that owners have a clear understanding of the complexities of diagnosis of PPID, including explaining seasonal variations in ACTH, and the difference between EMS and PPID and how these are diagnosed, as well as visible changes to look out for. The strong reliance on veterinary advice reported here means small improvements in communication by veterinary surgeons may have a large impact on treatment compliance.

Appendices for Chapter 7

Appendix 1: The invitation letter/email and consent form sent to and signed by all interviewees.

Dear *name of owner*,

Thank you for your participation so far in our research study. Further to our telephone conversation on Friday I am writing to confirm the time and date of our interview which will take place at ***address*** on **23/10/2018** at **2pm. Please can you just confirm the address we have on record is correct?**

The purpose of these interviews is to further understand the choices owners make when caring for a horse or pony with PPID (Cushing's), how owners understand their horse's health and wellbeing, and the different challenges that owners face. The information gathered will be used to help develop effective healthcare plans to improve the quality of life of horses and ponies with PPID.

During the interview I will ask questions about your experience of caring for Flynn and follow-up on topics touched on in the baseline questionnaire you completed.

The interview will take approximately **1.5 hours.** However, this will be very much led by yourself and it may take more or less time depending on the extent of our discussion. The interview will be audio recorded on a Dictaphone to ensure I do not miss anything you say.

Prior to this visit, please could you complete the attached consent form which you can return at the time of the interview. If you have any questions or would like to change the date or time of your appointment, please contact me by email at Rebecca.tatum@liverpool.ac.uk or call directly on 07467 374951.

Best wishes,

Becky

Becky Tatum, PhD Student, University of Liverpool
Dr Joanne Ireland, PhD Supervisor, University of Liverpool
Professor Catherine McGowan, PhD supervisor, University of Liverpool

Committee on Research Ethics

INTERVIEW PARTICIPANT CONSENT FORM

Title of Research Project:	Optimising Care for Cushing's (PPID) Horses and Ponies - Interview Phase	**Please initial in the box**
Researcher(s):	Becky Tatum (PhD Student), Dr Joanne Ireland, Prof Cathy McGowan and Dr Rachel Dean	
• I confirm that I have understood the information provided by the interviewer and in the appointment confirmation letter for this interview. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.		
• I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected. In addition, should I not wish to answer any particular question or questions, I am free to decline.		
• I understand that, under the Data Protection Act, I can at any time ask for access to the information I provide and I can also request the destruction of that information if I wish.		
• I understand that confidentiality and anonymity will be maintained and it will not be possible to identify me in any publications.		
• I understand and agree that my participation will be audio recorded and I am aware of and consent to your use of these recordings for the purposes.		
• I understand that my responses will be kept strictly confidential. I give permission for members of the research team to have access to my anonymised responses. I understand that my name will not be linked with the research		

materials, and I will not be identified or identifiable in the report or reports that result from the research.	
• The information you have submitted will be published as a report; please indicate whether you would like to receive a copy.	
• I agree to take part in the interview.	

Participant Name	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

Student Researcher:
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Appendix 2: The interview topic guide used during all interviews

Interview Guide

Introduction

- Thank you
- About 1.5 hour
- Taking notes and digitally recording
- Please speak clearly
- Confidential
- Information anonymised
- Do not have to talk about anything they don't want to
- Can take a break or stop at any time
- Any questions?

Background information

1. How has *name* been doing since we last spoke (to go through the baseline questionnaire)?
    - *Health?*
    - *Any changes?*

Management

2. Tell me about looking after your horse with Cushing's?
    - *Routine - Stabling/turnout/diet/feeding?*
    - *Are there particular reasons for the routine you have chosen?*
    - *How do you decide on changes to your horse's routine? Can you give me a recent example?*
    - *Is exercise a part in your horse's routine? If so, how often/doing what?*
    - *Do you feel that your horse is happy with his/her current set-up?*

3. How has being diagnosed with PPID affected his/her routine?
    - *Changes to diet/turnout/exercise compared to before diagnosis?*

Treatment

4. Can you tell me about your experience of treating your horse with Cushing's?
    - *Treatment given?*
    - *Treatment duration?*
    - *Did treatment make a difference to your horse?*
        - *Improved? Worsened?*
    - *How did you decide on the treatment your horse received?*
        - *Influencing factors?*
        - *Barriers?*
    - *Did you stop treatment at any time? If so were there any particular reasons for deciding to stop treatment?*

5. If he/she wasn't treated, were there any particular reasons for deciding not to use treatment?
    - *Not recommended?*
    - *Possible side effects?*
    - *Investigating alternative options?*

6. What is your opinion on pergolide/Prascend/prescribed tablets? (If not covered above)
    - *(if applicable) Can you tell me about your personal experience of using them?*

7. How do you feel about alternative treatments/therapies e.g. herbal supplements, homeopathy etc for the treatment of Cushing's?
    - *Why do/don't you use them?*

8. What do you think about the advice available to you regarding treating your horse with PPID?
    - *What made it clear/unclear?*
    - *Was it consistent?*
    - *What made you feel it was tailored to your horse/or not?*
    - *When were follow-up visits made/if at all?*
    - *Did you look online for advice? Was it helpful?*

9. Do you think anything could be done to improve the treatment of horses with Cushing's?
    - *Anything that could be done differently?*

Quality of life

10. What does the term quality of life mean to you?
    - *Familiar term?*
    - *What images does it conjure up?*

11. What do you think makes a good quality of life for your horse with Cushing's?
    - *What factors affect your horse's happiness and comfort?*
    - *What factors do you think are most important to ensure your horse has a good quality of life?*
    - *Does your horse have any other health problems? If so, how do these affect his/her quality of life?*
    - *What signs tell you your horse is in good health?*

12. What is more important, your horse's happiness or his/her health?
    - *Do you make changes to improve your horse's health which may affect his/her happiness or vice versa? (e.g. Eating chocolate is bad for us but we do it because we enjoy it)*

Concluding discussion

Anything further they wish to add?

Reiterate the confidential nature of the study and confirm they have contact details in case they have any further questions.

Appendix 3: Iterative process of mapping codes onto an A0 pinboard

Chapter 8

Overall discussion

Overall discussion

The studies presented in this thesis represent a novel mixed methods investigation into the health and QoL of horses with PPID in the UK, as well as the priorities of those that care for them and lay beliefs surrounding their management. The combination of qualitative and quantitative research methodologies used enabled a more rounded picture of the management and treatment of PPID to be presented. The initial JLA priority setting study is the first to introduce patient and public involvement (PPI) into equine veterinary medicine, identifying and prioritising uncertainties regarding the diagnosis, treatment and prognosis of PPID. The subsequent studies help to build on the evidence base and begin to fill some of the most important research gaps. The review chapters represent the first systematic knowledge synthesis to evaluate primary research regarding the most commonly used diagnosis and treatment protocols, providing a comprehensive assessment of current available evidence. Further to this, the two epidemiological studies are novel investigation into owner treatment and management of the disease, providing important information regarding owner management and treatment practices as well as the perceived QoL of their horses with PPID. Finally, the study around horse owner decision making is the first to use qualitative sociological methods to try and understand how lay beliefs affect the management and treatment of horses with a long-term disease.

The JLA priority setting partnership (Chapter 2) highlighted the many uncertainties which remain regarding the diagnosis, treatment and management of PPID and the lack of robust evidence regarding the disease. The JLA study also demonstrated the successful adaptation of PPI methodology into equine veterinary medicine. The views of end users are often overlooked in research fields (Whitney et al., 2006; Cowan, 2013). This process gave a voice to the veterinary surgeons who treat the animal and the owners who manage the disease on a daily basis, demonstrating the value they can add when identifying research priorities. However, there was a lack of engagement from veterinary surgeons in the rounds of online surveys. This may have been for a number of reasons, such as how the survey was disseminated, the open style of questions or the unfamiliar style of research. Veterinary surgeons in practice are time deficient: a survey of veterinary students described how time constraints were seen as the biggest barrier to being involved in research after graduation (Allan and Dunning, 2020). However, if the evidence base is to be improved effectively, they need to be encouraged to engage in PPI research. More widespread use of methodologies

such as the JLA framework could help to ensure that future research is more clinically relevant. Conversely, there was a good level of veterinary representation and input in the PSP workshop. This allowed owners and vets to share experiences and understand differing points of view. The kind of open discussion encouraged by PPI helps to bridge the gap between owners and veterinary surgeons and widen perspectives (Chalmers, 1995; Oliver, 1995; Entwistle et al., 1998), aiding the development of effectual relationships. The importance of the experiences and beliefs of end users such as veterinary surgeons and owners is being increasingly recognised in veterinary medicine with studies investigating both owner and veterinary decision making (Scantlebury et al., 2014; Bambra et al., 2017; Goyen et al., 2017; Belshaw et al., 2018; King et al., 2018). However, recognition of PPI methodology and the integration of this into the research process, such as seen in the NHS (NHS Executive, 2000; NHS Executive, 2004; Department of Health, 2013), needs to occur in order to see a switch from purely researcher led fields of investigation.

The cross-sectional study (Chapter 5) and prospective cohort study (Chapter 6) build on the evidence base available, filling some of the gaps prioritised in the JLA PSP. It is likely that the field-based sample recruited in Chapter 5 is more representative of the general veterinary diagnosed population of horses with PPID than previous small, predominantly hospital-based, populations studied. However, the method of dissemination meant it was not possible to calculate a response rate or to compare non-responders and limited the sample to owners who used the internet or social media. A subset of this sample registered with the UoLEP was then followed throughout the prospective cohort study (Chapter 6) and used to purposely select interview participants for Chapter 7. This meant the study population for both studies was limited to a single first opinion equine practice in the North West of England. Although telephone recruitment was used to maximise the sample in Chapter 6, non-response at the initial recruitment stage meant the number of horses included was smaller than anticipated. It was an accepted limitation of the study design that by using a convenience sample from a single veterinary practice means the study population may not be representative of the general population. However, inviting all eligible owners registered with a single veterinary practice reduces the potential variability of diagnostic and treatment recommendations, as may be seen between multiple veterinary practices. This aided the investigation of owner choices regarding daily management. Chapter 6 also used a combination of clinical records and owner questionnaires to obtain data, which provided a more comprehensive dataset than either route alone. However, this form of data collection

highlighted shortfalls in both approaches individually. There was some degree of recall bias or under-reporting by owners despite the short timeframe, which highlights the potential for inaccuracies in studies that use owner-reported data alone, such as Chapter 5. However, there were also deficiencies in clinical record keeping, which has possible implications for PPID research that relies solely on analysis of clinical record data, especially in retrospective studies.

The question prioritised as most important during the JLA process showed that end users want further certainty around the long-term prognosis of horses with PPID, and whether treatment influences how the disease ultimately progresses. A retrospective study analysed survival of chronic diseases and reported that although horses with PPID had a median life expectancy of nearly 10 years following diagnosis, the disease was negatively associated with survival ($p<0.01$) (Welsh et al., 2016). However, the influence of treatment was not investigated. It has been suggested that early intervention with pergolide treatment could help slow the progression of neurodegeneration (McFarlane, 2007), however the evidence around this is lacking. Two recent studies, conducted since the JLA priority setting partnership (PSP), have made some attempt to investigate the effect of treatment on survival of PPID. In a cohort of 137 cases, both treatment with pergolide and veterinary-reported clinical improvement were associated with increased likelihood of survival (both $p=0.01$) (Horn et al., 2019). However, this retrospective study was based in the southern hemisphere and had a high level of loss to follow-up. A further study followed cases for up to ten years and reported the majority of owners strongly agreed that treatment with pergolide improved their horses QoL (71%) (Schott et al., 2020b), however, this was based on smaller numbers. Chapter 6 prospectively followed PPID cases over a period of approximately 12 months and did not find any association between pergolide treatment and survival. This is likely to be due to the relatively small sample size and small number of untreated cases. PPID was not reported as the reason for mortality in any of the cases euthanased during the follow-up period ($n=11$), but was stated as a contributing factor to their decision by three owners. The most robust way of investigating any associations between treatment with pergolide and survival would be a randomised controlled trial, however, this has various ethical considerations, such as withholding a treatment which is known to be beneficial. Therefore, the findings of Chapters 5 and 6 could be built on in several ways, more in-depth analysis of both prospective and retrospective data would increase time at risk and allow further comparison of owner-reported information with

clinical records to assess disease progression. Furthermore, a large multi-centre cohort study could provide further insight into treatment efficacy and factors affecting long-term survival.

Chapter 5 demonstrated that the majority of owners (86.9%) administered pergolide, the treatment of choice for PPID. This corresponds with the 88.3% of horses receiving treatment in a recent Australian study (Horn et al., 2019). At the start of the study, the majority of owners (65.8%) reported that the pergolide dose administered had not changed in the preceding 12 months (Chapter 5). Similarly, the dose of pergolide remained largely unchanged in the prospective cohort study, with 78.2% of cases having no dose alterations throughout the follow-up period (Chapter 6). This suggests owners did not alter dose with season and that sustained clinical improvement was observed on the same dose for a prolonged period of time. Pergolide treatment was rated as highly effective by owners, with a median efficacy rating of 8/10 (IQR 7-10) and has been shown to be effective at resolving ≥1 clinical sign in the majority of cases (≥76%) (Chapter 4). Chapter 5 built on this by demonstrating that pergolide treatment was associated with improved QoL ($p=0.008$). This is in line with the various studies that demonstrate clinical improvement following pergolide treatment (Chapter 4). However, despite this high efficacy rating, just over half of owners who treated their horse with pergolide reported observing side effects during the first two weeks of treatment (56.5%) and side effects were the predominant reason for stopping treatment (Chapter 5). It is therefore unsurprising that veterinary surgeons and owners ranked the question around side effects sixth during the JLA PSP. The most frequently reported side effects in Chapter 5 were loss of appetite (31.5%), lethargy/poor performance (21.0%) and diarrhoea/loose faeces (6.0%). A few studies in the systematic review of pergolide efficacy (Chapter 4) also reported prevalence of side effects, the most robust of which reported 33% of horses developed transient inappetence (Anon, 2011a). This was reported resolve following to a short-term dose reduction (Anon, 2011a). The higher prevalence of side effects reported by owners in Chapter 5 may be as a result of how well owners know their horse and the level day to day interaction they have with them (Chapter 7) or a failure to reduce the dose of pergolide promptly at the onset of side effects. Veterinary surgeons need to ensure they clearly communicate with owners when commencing treatment to ensure they are aware of how to deal with common side effects which can be easily resolved if addressed quickly. The communication approach of veterinary surgeons can have huge influence on owner response and compliance,

consequently impacting the success of treatment (Lue et al., 2008). Therefore, effective communication is likely to help to reduce the number of cases where treatment is stopped.

Pergolide's effect on the occurrence of laminitis was of particular interest to end users (Chapter 2). Chapter 6 investigated the incidence of laminitis among both treated and untreated cases and found a lack of treatment effect, with six of the eight animals having at least one episode of laminitis during the follow-up period reported to be receiving pergolide treatment. This is similar to the finding of another recent study which found no association between treatment and a diagnosis of laminitis (Horn et al., 2019). This is likely to be because the underlying cause of laminitis in PPID cases is concurrent insulin dysregulation rather than PPID itself (Karikoski et al., 2016; de Laat et al., 2019a,b), therefore effectively treating PPID alone without addressing insulin dysregulation may not be sufficient to lead to an improvement in laminitis. It was beyond the scope of the studies presented here to undertake further investigate into the effect of pergolide on individual clinical signs. Therefore, additional investigation is required into this area as well as what other options could be considered in the ≤24% of cases (Chapter 4) where clinical signs do not satisfactorily resolve.

Owners perceived the majority of horses to be enjoying a very good QoL (median rating = 9/10; IQR 8-10) (Chapter 5), similar to that described for the general geriatric population (Ireland et al., 2011c). This QoL rating was based on owner rated overall QoL. Although various individual QoL domains were also assessed, such as activities of daily living and demeanour, direct associations between these domains and overall QoL was not assessed. This is an area which warrants further investigation to establish effective QoL ratings for use in horses. Pergolide treatment was also associated with an improvement in QoL rating since the time of diagnosis ($p=0.002$), with 75.9% (n=203/303) of owners reporting an improvement in their horse's QoL following treatment. This is in keeping with the overall improvement in clinical signs observed in the majority of cases (≥76%) across treatment studies (Chapter 4, Figure 2) as well as the proportion of horses deemed to be treatment successes in a large field trial (76%) (Anon, 2011a). This high QoL appeared to be maintained over time during the follow-up period (Chapter 6), however, during follow-up a number of clinical signs were reported to persist and some owners reported that their horses exhibited new ones. This indicates that owners still perceive their horse to have a good QoL when they are demonstrating signs of PPID, suggesting that clinical presentation

is not necessarily seen by owners as directly linked to QoL. This has also been observed in human medicine with the association between clinical variables and QoL domains varying from 12-40% (González-Blanch et al., 2018). The link between various clinical signs and QoL is an area which would benefit from further exploration, however the studies presented here go some way to demonstrating that specific clinical signs were negatively associated with QoL. In Chapter 7, owners described how health and happiness go 'hand in hand' when it comes to QoL. Seeing their horse as pain free was an important factor in this and was mainly visualised through their horse's demeanour. Clinical signs such as coat changes are unlikely to be associated with pain and therefore may not be perceived to affect QoL, resulting in the high rating reported. However, several clinical signs, such as current active laminitis, muscle wastage and lethargy/poor performance, were negatively associated with QoL (Chapter 5). This is because these signs are more likely to be linked with pain and a change in demeanour, as well as affecting the horse's day to day life, and therefore are recognised as a deviation from the norm by owners (Chapter 7). Conversely, QoL from a veterinary perspective is likely to be much more objective and focussed on health-related QoL, therefore more closely linked to the clinical signs demonstrated. Further research is required to investigate veterinary perceptions of QoL and how this differs from owner perceptions.

Two questions in the JLA top 10 (Chapter 2) centred around diagnostic accuracy, demonstrating that further clarity is needed regarding diagnostic protocols. Protocol recommendations have been made for the commonly used basal ACTH test (EEG, 2019). However, Chapter 3 is the first to systematically review the current available evidence regarding the diagnostic accuracy of this primary test using standardised criteria. The basal ACTH test was found to be relatively accurate with a median reported sensitivity of 75.5% (range 36.0-100%) and specificity of 95.2% (range 63.3%-100%) (Chapter 3). However, this systematic review also highlighted that evidence is predominantly based on small studies with poor or inconsistent methodology and a high risk of bias. This meant meta-analysis was not undertaken, as it would likely have compounded or exaggerated the biases highlighted. Therefore, although the data synthesis presented in Chapter 3 builds on the evidence available regarding the accurate diagnosis of PPID, the primary evidence base needs to be improved before meta-analysis can be undertaken and stronger conclusions drawn about the diagnostic accuracy of basal ACTH.

The question ranked second pertained to the various in vivo and in vitro factors that are reported to effect ACTH levels and therefore diagnostic test accuracy. Studies have described how factors such as stress caused by travel (Fazio et al., 2008) and treadmill exercise (Nagata et al., 1999) can increase ACTH levels. It has also been reported that pain can lead to an increase in the release of ACTH and cortisol from the pars distalis (Molony and Kent, 1997; Beech et al., 2007; Gehlen et al., 2020). Pain is of particular interest in the diagnosis of PPID due to comorbidities such as laminitis. Laminitis was the most frequently reported reason that owners suspected PPID (28.6%) (Chapter 5) and the overall prevalence of laminitis in PPID cases has been reported to be 48.9% (Ireland and McGowan, 2018). Therefore, there is some potential that pain may influence test results at diagnosis and throughout monitoring in a considerable proportion of cases. It was beyond the scope of the review in Chapter 3 to investigate individual factors which affect the reliability and accuracy of the basal ACTH test. A further scoping review is needed to provide clarification regarding the extent to which these factors may affect diagnosis of PPID.

Further uncertainty was around inconclusive or conflicting test results and/or clinical signs (question ranked eighth; Chapter 2). The lack of a consistent association between clinical signs and ACTH levels was highlighted in Chapter 4, meaning ACTH levels may not necessarily reflect the clinical picture. The theme 'disease tangibility' in Chapter 7 demonstrated how this can cause confusion and resistance among owners. ACTH test results were a way of quantifying the disease and a factor on which treatment decisions are based. Therefore, when they did not match the horse they were seeing in front of them, owners found the diagnosis difficult to understand. Their horse needed to be exhibiting what were seen as the 'typical signs'. Owners were looking for these 'typical signs' and the effect on their horse's day to day life to realise the disease and ultimately base management and treatment decisions around. Therefore, owners are unlikely to recognise the disease unless there is an obvious change in how their horse looks or acts. This finding has been reflected in other studies where owners under-recognise clinical signs or do not attribute them to disease (McGowan et al., 2010b; Ireland et al., 2012a). This shows the challenge of recognising PPID, even before the stage of laboratory testing is reached. Two of the main reasons for owners suspecting PPID were veterinary suggestion and an overgrown coat (25.7% and 23.3%, respectively) (Chapter 5). This suggests that diagnoses in the earlier stages of disease may be coincidental when the veterinary surgeon is attending for another reason. Therefore, although further research is undoubtedly needed to understand the

relationship between the increased circulation of POMC-derived peptides and the clinical signs of PPID, just as important is the education of owners regarding the progressive and varied nature of the disease. Increasing owners' awareness and ability to recognise and monitor more subtle clinical changes could improve management and treatment decisions, as well as the chances of early diagnosis.

The prioritised questions ranked third and fourth related to additional treatment and management options for horses with PPID (Chapter 2). This suggests that although pergolide is considered to be an effective treatment, there is still a need for further management strategies to be investigated. The reported management of horses with PPID in Chapter 5 was not dissimilar to that described for the general geriatric population (Brosnahan and Paradis 2003a; Ireland et al., 2011a), with the exception of access to grazing. History of laminitis was significantly associated with a reduction in hours turned out throughout the year ($p=0.003$) and restricted grazing was associated with decreased QoL rating ($p=0.006$). Conversely, horses turned out at grass were perceived to have a significantly better QoL ($p=0.007$). Laminitis was a major influencer on how owners perceived PPID, irrespective of their experience of it. It was one of the main concerns and considerations when it came to making management decisions (Chapter 7). This is unsurprising considering the painful nature of laminitis and the risk of recurrence (Potter et al., 2017; de Laat et al., 2019a). When owners described balancing management complexities, perceived risk of laminitis was the main consideration and a key factor in practices such as restricting grazing. However, when access to grazing was restricted, it diminishes the amount of freedom horses have to exhibit natural behaviours while turned out, which was an important part of the horse centred approach described in Chapter 7. Similarly, a study investigating stakeholder perceptions of racehorse welfare identified opportunities for the horse to socialise and be as 'natural as possible' as part of a 'best life' model (Butler et al., 2019). The other key influencer on management was personal experience, with 67.2% of owners basing decisions regarding diet predominantly on their own personal experience (Chapter 5). This was also reflected in Chapter 7, where owners considered themselves experts in their own horse and were therefore able to determine what was best for them on a day-to-day basis.

The effectiveness of non-prescription or alternative treatments was encompassed in the question raked forth during the JLA. This was a controversial topic which created much

debate during PSP workshop (Chapter 2). Only a small proportion of horses were receiving alternative treatments (8%) and these were mainly given as complementary therapy alongside pergolide treatment (Chapter 5). These treatments had a lower efficacy rating (median = 5/10; IQR 2.5-7) and were associated with a lower QoL rating (p=0.003) compared to pergolide treatment alone. Despite this lack of efficacy, their use could be for a multitude of reasons. In human medicine it has been reported that a poorer health status is associated with the use of alternative medicine (odds ratio 1.3) (Astin, 1998), while another study reported that worries about the cost of treatment were associated with individuals being more likely to use complementary or alternative medicines (Barnes et al., 2008). Owners may use complementary or alternative treatments when sufficient improvement had not been observed with pergolide alone. Their use could be seen as a way of reducing pergolide dose and therefore cost or as a low-cost alternative in horses that are showing early or mild clinical signs. Cost of treatment was a consideration highlighted in Chapter 7, particularly because of the need to administer it long term. However, this tended to be overridden by the strong relationship that owners have with their horse and their want to do the best for their horse described in Chapter 7. This perhaps accounts for the small proportion of cases who did not receive pergolide treatment in Chapters 5 and 6.

The vet-owner relationship has been shown to be an important influencer when it comes to owner's treatment decisions. Studies investigating barriers to the uptake of Hendra virus vaccination in Australia found that a negative perception of veterinary surgeons hindered the uptake of vaccination (Goyen et al., 2017; Manyweathers et al., 2017). In small animal medicine, trust and communication have been shown to be important factors in building relationships with owners (Brown, 2018). Similarly, trust was an important factor in the vet-owner relationship theme described in Chapter 7. When it comes to the treatment of PPID, owners generally described having a good relationship with their veterinary surgeon (Chapter 7), and this was also reflected in Chapter 5 where 81.4% of owners stated that advice from their veterinary surgeon influenced their treatment decisions. However, decisions are also influenced by a complex mixture of other factors such as the owner's individual experiences and knowledge of their horse as well as wanting to feel in control of the treatment and management strategies being undertaken (Chapter 7). This novel insight into owner decision making and perceptions of equine veterinary surgeons can be used to advise veterinary surgeons when communicating with owners. A more diverse sample selected from a variety of practices is needed to provide a fuller picture of the vet-owner

relationship. However, the findings of Chapter 7 demonstrate that veterinary surgeons need to remember the owner behind the horse and that communicating effectively with them is just as important as treating the animal.

Conclusion

Overall, the work presented here has helped to begin filling the evidence gaps highlighted in the JLA PSP. It builds on the evidence base in a way which is relevant and useful to end users by addressing the areas most important to them. The systematic reviews offer evidence about diagnosis and treatment of PPID which is much higher on the hierarchy of evidence than any of the individual studies reviewed. These will help facilitate an evidence based veterinary medicine approach by improving the evidence available and providing a 'one stop' source that busy clinicians can incorporate with clinical judgement. Chapter 3 will contribute to a better understanding of the use of basal ACTH for diagnosing PPID while Chapter 4 will enable veterinary surgeons to be confident in recommending pergolide treatment. Chapters 5 and 6 build on the primary evidence base, giving insight into the management practices and perceptions of owners. Providing useful information for veterinary surgeons when advising owners regarding ongoing management outside of veterinary visits. Finally, Chapter 7 emphasises the importance of understanding owner perspectives and the need for veterinary surgeons to communicate effectively with owners regarding treatment approaches. In order to be most effective, treatments should be administered as prescribed, and this chapter demonstrates how veterinary surgeons need to remember that building an effective relationship with the owner behind the horse, communicating with them successfully regarding management and treatment approaches is just as important treating the animal itself.

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