Comparing Treatment Methods of Canine Hypoadrenocorticism

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A thesis submitted in fulfilment of the requirements for the Degree of

Master of Veterinary Medicine

of the

School of Veterinary Medicine

College of Medical, Veterinary & Life Sciences

University of Glasgow

March 2020

Abstract

Management of canine hypoadrenocorticism relies on supplementation of glucocorticoids and mineralocorticoids. Although previous studies show success of both a steroid with combined activity (fludrocortisone) and steroids with separate activities (DOCP with prednisolone) in the management of canine hypoadrenocorticism, the two treatment methods had never been prospectively compared.

The objective of this clinical trial was to compare fludrocortisone to DOCP with prednisolone for the management of canine hypoadrenocorticism in stable patients.

A prospective, randomised, cross-over, non-blinded, non-inferiority trial was conducted. Patients were randomised into two groups: Group A received three months of treatment with the interventional product (DOCP and prednisolone) followed by three months of the control product (fludrocortisone) whilst Group B received three months of the control product followed by three months of the interventional product. Primary outcome measures were electrolyte concentrations and clinical signs at the end of each phase of the trial. Secondary outcome measures included plasma renin activity, endogenous ACTH, blood pressure and routine haematology/biochemistry results.

No dogs had clinical signs of hypoadrenocorticism or hyponatraemia/hyperkalaemia at the end of the DOCP phase of the trial. Three dogs however were hyponatraemic at the end of the fludrocortisone phase of the trial, although no patients were hyperkalaemic or showed clinical signs of hypoadrenocorticism. The blood pressure was significantly higher (Paired T test; P=0.006) at the end of the DOCP phase (mean 161mmHg; SD 26.3) than at the end of the fludrocortisone phase (mean 147mmHg; SD 26.2). The neutrophil count was significantly higher (Paired T test; P=0.009) at the end of the DOCP phase (mean 7.49x10^9/L; SD 3.1) compared to the fludrocortisone phase (mean 5.81x10^9/L; SD 2.31). The urea was significantly lower (Wilcoxon Signed Rank test; P <0.001) at the end of the DOCP phase (median 5.3mmol/L; 2.9-11.7) compared to the end of the fludrocortisone phase (median 7.4mmol/L; 3.8-17.2). The creatinine was also significantly lower (Paired T test; P=0.001) at the end of the DOCP phase (mean 96umol/L; 19.2) compared to the fludrocortisone phase (mean 109umol/L 28.9). The ACTH and renin concentrations were significantly lower at the end of the DOCP phase compared to the fludrocortisone phase (Wilcoxon Signed Rank test; both P <0.001).

In conclusion, DOCP with prednisolone appears to be non-inferior to fludrocortisone acetate for the management of canine hypoadrenocorticism.

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Dedication

I would like to dedicate this thesis to Maddie and Benji: the Tibetan Terriers who taught me more than the lectures, books and journals. They let me experience how much someone can love an animal. They taught me that pets are family members. They showed me how difficult their loss can be. They made me confident that I wanted to be a vet and allowed me to be a vet with empathy, not sympathy. More specifically, with their hyperadrenocorticism, hypothyroidism and diabetes mellitus between them, they ignited my passion for endocrinology before I even arrived on the doorstep of Glasgow Veterinary School.

Declaration

I, Susanna Spence, declare that the work in this thesis is original, was carried out solely by myself or with due acknowledgements. It has not been submitted in any form for another degree or professional qualification at the University of Glasgow or any other institution.

Printed Name: Miss Susanna Spence

Signature: ……..

Acknowledgements

I would like to thank my primary supervisor Professor Ian Ramsey at the School of Veterinary Medicine, University of Glasgow, for his support and guidance throughout this study: from the study design to the review of my thesis. I would also like to thank my second supervisor Mrs Alix McBrearty, also of the University of Glasgow School of Veterinary Medicine for her support in the writing of this thesis.

I would like to specifically thank Mr Samuel Fowlie and Ms Emma Roberts, the veterinary surgeons involved in the trial along with myself and Professor Ramsey. They invested a great deal of their time in the trial to ensure it ran smoothly and that the study protocol was strictly adhered to. I would also like to thank the Internal Medicine team at the University of Glasgow Small Animal Hospital for their assistance in the trial and in particular the Internal Medicine nurses Mrs Sharon Smith, Miss Kelly Cadman and Mrs Susan McKenzie.

I am very grateful for the support from the Clinical Pathology Laboratory at the University of Glasgow Veterinary School for running our samples in a timely fashion. In addition, I would like to acknowledge Mrs Helen Evans at Nationwide Specialist Laboratories for assistance with the measurement and interpretation of the plasma renin activity and endogenous ACTH assays.

I would also like to acknowledge all 33 dogs enrolled on the trial and their owners who gave so much of their time to attend regular appointments at the Small Animal Hospital, University of Glasgow. In addition, thanks must go to the referring veterinarians who assisted in recruitment of these patients and allowed us to take over their management for six months.

Last but certainly not least, I would like to thank all the staff at Dechra for their financial and administrative support. In particular I would like to acknowledge Mrs Hayley Farr, Mrs Beasley Mason and Mr Greg Williams for their input. Thank you to Dr Kerstin Adler of Klifovet, The Study Monitor, for her numerous trips to Glasgow to ensure compliance with the trial protocol.

1 Introduction: Review of the literature on canine hypoadrenocorticism and its management

1.1 The Adrenal Gland

1.1.1 The Anatomy of the Adrenal Gland

The adrenal gland is a yellow-white organ situated medial to both kidneys, lateral to the renal vessels and dorsal to the vena cava. They are flattened dorsoventrally and in dogs the normal gland is around 2-3cm long and 1cm wide (Singh and Dyce, 2018). Adrenal gland size is not necessarily correlated with body weight or breed in dogs (Juodžiukynienė et al., 2014). The adrenal gland can be separated into distinct regions both histologically and functionally. Anatomically, the adrenal gland consists of an inner medulla and an outer cortex. The cortex can then be separated into 3 layers: the outer zona glomerulosa (25%) which comprises columns of cells in a sigmoid arrangement; the middle zona fasciculata (60%) which contains cells with cytoplasm abundant in lipid and are surrounded by numerous small capillaries (Rosol and Grone, 2016) and the inner zona reticularis (15%) which contains numerous secretory cells surrounded by capillaries (Van Lanen and Sande, 2014). Ultrasonographically, the adrenal glands are located cranial and medial to each kidney, with the phrenicoabdominal vein running ventral to the gland and the phrenicoabdominal artery coursing dorsally. Normal adrenal glands are contained within a small hyperechoic capsule with a hypoechoic, homogenous centre (Barthez et al., 1998). The right adrenal gland is more difficult to locate given its more cranial position. Left adrenal gland thickness <3.2mm is highly suggestive of hypoadrenocorticism (Wenger et al., 2010).

1.1.2 The Physiology of the Adrenal Gland

The main role of the adrenal gland is to produce hormones. The adrenal medulla produces catecholamines whilst the adrenal cortex produces glucocorticoids, mineralocorticoids and androgens (Cunningham and Klein, 2013). In hypoadrenocorticism, the hormones of most significance are cortisol and aldosterone.

1.1.2.1 Cortisol Production and Action

Production of cortisol (a glucocorticoid) from cholesterol is complex, involving numerous enzymes and is reviewed elsewhere (Auchus and Miller, 2016). Control of glucocorticoid release is regulated by the hypothalamic-pituitary axis (HPA). Corticotrophin (CRH) is released from the hypothalamus and activates receptors on the anterior pituitary gland, stimulating the release of adrenocorticotrophic hormone (ACTH). This hormone then travels via the blood to the adrenal cortex where it stimulates cortisol production and secretion from the zona fasciculata and reticularis. Cortisol has a negative feedback effect on both CRH and ACTH release, therefore self-regulating its control. In other species such as humans, ACTH concentrations fluctuate with circadian rhythm. This is not the case in dogs however ACTH can be stimulated by arginine vasopressin (AGP), angiotensin II, cholecystokinin (CCK), atrial natriuretic factor (ANP) and vasoactive peptides, irrespective of cortisol concentrations. In addition, ACTH concentrations can be affected by stress and hypoglycaemia, secondary to their stimulation of CRH release (Scott-Moncrieff, 2015).

Glucocorticoids have a vast number of actions and they are an important mediator of physiological stress. Glucocorticoids also stimulate gluconeogenesis and glycolysis as well as protein and fat catabolism. They maintain intestinal cell integrity and are important in digestion and absorption within the gastrointestinal tract (Langlais-Burgess et al., 1995). Although cortisol does bind (with as much infinity) to mineralocorticoid receptors, it does not have as potent mineralocorticoid effects as it is readily de-activated to cortisone in the collecting ducts where these receptors are found (Scott-Moncrieff, 2015).

1.1.2.2 Aldosterone Production and Action

The zona glomerulosa is the only layer of the adrenal cortex which is capable of secreting aldosterone. This is because it is the only region containing the enzyme aldosterone synthase (P450 c11AS) which is required for its synthesis. In addition, the zona glomerulosa does not contain 17-alpha-hydroxylase (P450 c17) and therefore, cannot synthesise cortisol (Scott-Moncrieff, 2015).

The secretion of aldosterone is stimulated by angiotensin II and increased plasma potassium concentrations (and to a lesser extent, decreased sodium concentrations). Aldosterone causes upregulation of the sodium/potassium ATPase pump in the distal convoluted tubule of the kidney, which results in reabsorption of sodium and excretion of potassium. As water moves with sodium, this results in increasing the circulating plasma volume and therefore, blood pressure. Chloride is also absorbed via the paracellular pathway and sodium can be absorbed by the collecting tubule cells in exchange for hydrogen ions (Cunningham and Klein, 2013). This regulation of blood pressure is controlled by the renin-angiotensin-aldosterone system. Renin is released from the macula densa of the juxtaglomerular apparatus of the kidneys (at their glomeruli) in response to reduced sodium concentrations or extracellular fluid volume. This results in the conversion of angiotensin to angiotensin I in the liver. Angiotensin I is then converted to angiotensin II in the lungs, before stimulating aldosterone release from the zona glomerulosa. The increased sodium concentrations achieved following aldosterone release result in reduced release of renin. When ACTH is completely absent, reduction in aldosterone secretion is seen, however complete hypophysectomy does not result in low aldosterone concentrations(Rauschkolb et al., 1956). Therefore, ACTH is thought to have a minimal effect on aldosterone release.

1.2 Causes of hypoadrenocorticism

1.2.1 Primary Hypoadrenocorticism

Primary hypoadrenocorticism is caused by bilateral destruction of the adrenal cortices. Clinical signs develop once 90% or more of the relevant adrenal cortex zona is non-functional (Boag et al., 2015, Bellumori et al., 2013, Boujon et al., 1994). In over 95% of dogs, the condition is idiopathic and suspected to be due to immune mediated destruction of the gland. Histologically affected glands have lymphocytic infiltrate (Boujon et al., 1994) with a lesser population of plasma cells and macrophages. The predominant population is CD 4+ T cells and there is also histological evidence of replacement of the glandular tissue with fibrous tissue in the later stages of the disease, as the glands atrophy (Friedenberg et al., 2016). In humans, this autoimmune disease is caused by circulating autoantibodies against 21-hydroxylase11 and p450 side chain cleavage and more recently this has been demonstrated in some dogs (Boag et al., 2015, Boag and Catchpole, 2014).

In classical primary hypoadrenocorticism, destruction of the zona glomerulosa and fasciculata are seen, resulting in both mineralocorticoid and glucocorticoid deficiency. However, “atypical hypoadrenocorticism” describes a subset of patients who present with glucocorticoid deficiency only (Sadek and Schaer, 1996, Thompson et al., 2007). These patients have only destruction of their zona fasciculata.

There are many clinical manifestations of glucocorticoid deficiency. These include hypoglycaemia due to reduced gluconeogenesis and glycogenolysis and reduced protein and fat metabolism, resulting in weakness, weight loss and muscle wastage (Peterson et al., 1996). Patients with glucocorticoid deficiency also have a reduced ability to cope with physiological stress or infectious stimuli. Without the effects on gastrointestinal cell and brush border regeneration, diarrhoea can be seen however other gastrointestinal signs such as vomiting and inappetence can also be seen and this is thought to be multi-factorial. Causes of gastrointestinal signs include reduced peristalsis, poor intestinal absorption, hypovolaemia, poor perfusion and vascular stasis. These effects in turn can cause gastrointestinal ulceration and blood loss (Thompson et al., 2007).

Deficiency of aldosterone leads to hypovolaemia and clinically, to dehydration. It can also lead to hyperkalaemia which results in bradycardia and conduction abnormalities of the heart. With aldosterone deficiency, a mild metabolic acidosis can also be seen given the lack of excretion of hydrogen ions in exchange for sodium ions (Scott-Moncrieff, 2015).

Numerous other causes of adrenal gland atrophy (primary hypoadrenocorticism) have been reported. Neoplastic causes can be primary neoplasia such as lymphoma or metastatic neoplasia (Buckley et al., 2017). Other than lymphoma, it is not common for primary tumours to affect both glands (Kook et al., 2010) and therefore metastatic neoplasia is more common, with 21% of disseminated metastasis affecting these glands (Scott-Moncrieff, 2015). The most common tumours to metastasise to an adrenal gland include pulmonary, mammary, prostatic and pancreatic carcinomas (Labelle and De Cock, 2005). Infectious causes include fungal conditions (including blastomycosis, histoplasmosis and cryptococcosis) which cause granulomatous destruction of the adrenal gland (Scott-Moncrieff, 2015). The most common cause of primary hypoadrenocorticism, other than idiopathic destruction of the gland, is iatrogenic destruction caused by drugs such as trilostane and mitotane which are used in the management of hyperadrenocorticism (Reusch et al., 2007, Vilar and Tullner, 1959, Reid et al., 2014, Ramsey et al., 2008). Sporadic reports of primary hypoadrenocorticism include a report of 6 Beagles exposed to aerosols of plutonium-238 (Weller et al., 1996).

1.2.1.1 Signalment

Several breeds are thought to be over represented in populations of dogs with primary hypoadrenocorticism (Summers et al., 2010, Bellumori et al., 2013). In addition, several breeds have been recognised as being genetically predisposed including: Portuguese Water Dogs (Chase et al., 2006, Oberbauer et al., 2006); Nova Scotia Duck Tolling Retriever (Burton et al., 1997, Hughes et al., 2010, Hughes et al., 2007, Hughes et al., 2011); Standard Poodle (Shaker et al., 1988, Famula et al., 2003, Friedenberg et al., 2017); and Bearded Collie (Oberbauer et al., 2002). The condition has autosomal recessive heritability in the former three breeds but the heritability is unclear in the Bearded Collie (Scott-Moncrieff, 2015). In these breeds, several genes have been associated with increased susceptibility to hypoadrenocorticism. Some of these genes overlap with those found in humans with hypoadrenocorticism. Despite considerable research, the genetics of the susceptibility to hypoadrenocorticism is still poorly understood and no genetic or serological tests are currently available. Although no large scale genetic studies have been carried out, there is also anecdotal evidence for increased incidence of the condition in Soft Coated Wheaten Terriers (Haviland et al., 2016), West Highland White Terriers, Great Danes and Cocker Spaniels (Short et al., 2014, Short et al., 2013). It is suspected that there are variations and overlap between

the mechanism of autoimmunity between breeds and perhaps between individual patients (Boag and Catchpole, 2014).

Although patients can present with hypoadrenocorticism at any age (range 4 weeks to 16 years), they are usually young to middle aged with a median age of 4 to 5 years (Klein and Peterson, 2010a, Kintzer and Peterson, 1997a). Female dogs are over-represented at 69% of the total dog population (Hanson et al., 2016). There are however breed variations, for example, Nova Scotia Duck Tolling Retrievers present at a younger median age of 2.6 years and in this breed, Bearded Collies and Poodle breeds, there appears to be equal sex distribution (Oberbauer et al., 2006, Oberbauer et al., 2002, Famula et al., 2003). In addition, it has been suggested that neutered dogs are three times more likely to develop the condition compared to entire dogs (Kelch et al., 1998).

1.2.2 Secondary Hypoadrenocorticism

Spontaneous secondary or tertiary hypoadrenocorticism is rare. It is caused by reduction in ACTH or CRH from the hypothalamus and pituitary gland respectively. This has been reported in humans due to neoplasia or traumatic lesions, however this has not been reported in the dog. The only known cause of spontaneous secondary hypoadrenocorticism in the dog is pituitary apoplexy which can result in neurological signs and adrenal crisis (Bertolini et al., 2007). More commonly, secondary hypoadrenocorticism is caused by sudden withdrawal of glucocorticoids following long term use at doses higher than the physiological dose. This chronic glucocorticoid therapy causes reduced necessity for ACTH release from the pituitary gland and therefore transient anterior pituitary and adrenal gland atrophy can occur (Pey et al., 2012). ACTH suppression can occur any time from a few days after initiation of steroid therapy, however individual response to exogenous corticosteroids is variable (Scott-Moncrieff, 2015). Less commonly, iatrogenic secondary hypoadrenocorticism can be caused by hypophysectomy for management of pituitary dependant hyperadrenocorticism (Hanson et al., 2005) or medical management of pituitary or adrenal dependant hyperadrenocorticism (den Hertog et al., 1999, Alenza et al., 2006, Reid et al., 2014, Ramsey et al., 2008).

1.3 Investigation of hypoadrenocorticism

1.3.1 Historical and clinical examination findings in dogs with hypoadrenocorticism

1.3.1.1 History and Clinical signs

Hypoadrenocorticism is an uncommon condition with a prevalence of 0.06-0.28% in one referral hospital population in the United States of America (Kelch et al., 1998). Patients present with variable signs and urgency to clinicians. Clinical signs are vague with the most common being lethargy (67-76%), followed by anorexia (52-73%), vomiting (62-68%) and diarrhoea (29-56%) (Haviland et al., 2016, Wakayama et al., 2017). Less common clinical signs include polyuria/polydipsia (8%), weight loss (8%), generalised weakness (3%) and gait abnormalities (2%) (Haviland et al., 2016). Rare clinical signs have been reported such as regurgitation (Whitley, 1995), muscle cramps (Saito et al., 2002) and seizures (Van Lanen and Sande, 2014). The history of these signs can range from days to years, depending on the rate of adrenal gland atrophy and the patient's ability to compensate (Scott-Moncrieff, 2015). Generally, but not always, dogs with atypical hypoadrenocorticism tend to have a more insidious onset of disease (Wakayama et al., 2017). Often patients have waxing and waning signs or disease that is the sequel to a stressful event (Scott-Moncrieff, 2015). They may also have a history of re-occurring signs which respond to symptomatic therapy such as with fluid therapy (Scott-Moncrieff, 2015).

1.3.1.2 Physical examination findings

As with historical findings, physical examination findings are vague and some patients may have no abnormalities on physical examination. Findings can be attributed to the underlying glucocorticoid or mineralocorticoid deficiencies. One of the most common clinical findings is dehydration, which is due to the inability to conserve sodium in the distal convoluted tubules and collecting ducts of the kidneys (Scott-Moncrieff, 2015, Van Lanen and Sande, 2014). This hypovolaemia results in a reduction in blood pressure, with median systolic blood pressures being reported to be low at 90mmHg (Seth et al., 2011). Clinically dehydrated patients that do not have hypoadrenocorticism normally present with compensatory tachycardia and therefore, dehydrated patients with a normal or low heart rate (or even bradycardia) should prompt consideration of hypoadrenocorticism (Van Lanen and Sande, 2014). In these patients, the bradycardia is secondary to hyperkalaemia which results from the inability to excrete potassium from the kidneys. This change can also result in arrhythmias or muscle cramping (Saito et al., 2002).

Another common clinical finding is muscle wastage which is due to an inability to metabolise and utilise energy adequately and this can also result in weakness (Hughes et al., 2007). Patients can occasionally develop melaena or haematochezia due to poor enterocyte regeneration and ischaemic injury due to hypovolaemia. This can lead to anaemia which results in pale mucous membranes on examination (Scott-Moncrieff, 2015).

Given the vague findings on history and physical examination, there are numerous differential diagnoses for hypoadrenocorticism. These include gastrointestinal disease (such as inflammatory bowel disease), renal disease, hepatic disease and other endocrinopathies such as diabetic ketoacidosis. It is not uncommon for patients to have exploratory laparotomies performed to assess for obstructed foreign material within the gastrointestinal tract. Therefore routine investigations are required to exclude other causes of the vague signs and to increase the clinical suspicion of this condition, before performing confirmatory testing.

Historical and physical examination findings are discussed in more detail in other sources (Spence et al., 2018, Ettinger et al., 2017, Scott-Moncrieff, 2015, Mooney and Peterson, 2012, Hess, 2018, Church, 2012).

1.3.2 Clinical pathological changes associated with hypoadrenocorticism

1.3.2.1 Haematology

The most common haematological abnormality seen with hypoadrenocorticism is the absence of a stress leukogram in a sick patient. A reverse stress leukogram is represented by low neutrophils with a lymphocytosis and eosinophilia. Only 10 to 30% of patients with hypoadrenocorticism have a reverse stress leukogram, however over 90% has lack of a stress leukogram (Hess, 2018). In normal circumstances, cortisol causes lymphocytes to remain in the bone marrow and lymph nodes instead of being released, due to sequestration. In hypoadrenocorticism, the lack of cortisol prevents this from happening and therefore circulating lymphocytes increase (Scott-Moncrieff, 2015). Lymphocyte counts have been used to assist the diagnosis of hypoadrenocorticism with lymphocyte counts <0.75x10^3/µL excluding the condition (Seth et al., 2011). Neutropenia is less commonly seen than lymphocytosis and most commonly neutrophils are in the normal reference range. This is an uncommon finding in sick patients and up to 32% of patients with hypoadrenocorticism can have a neutrophilia (Hess, 2018). Eosinophilia is seen more commonly than lymphocytosis (20%) but a high eosinophil count is not as sensitive for detecting the condition (Chastain et al., 1989, Seth et al., 2011).

Another common haematological finding is a normocytic, normochromic, non-regenerative anaemia. This is primarily due to a lack of red blood cell production in the bone marrow due to cortisol deficiency, however it can also be in part due to gastrointestinal blood loss. Up to 15% of dogs are thought to have melaena and this can result in severe anaemia (Scott-Moncrieff, 2015). Often the anaemia can be masked by severe dehydration at the time of presentation, not becoming apparent until fluid resuscitation has been commenced. Generally, the anaemia is more marked with patients affected by atypical hypoadrenocorticism as the condition is not diagnosed as early due to a milder clinical presentation (Thompson et al., 2007)..

1.3.2.2 Electrolytes

The clinicopathological finding most classically associated with hypoadrenocorticism is a derangement in electrolyte concentrations. Hyperkalaemia is the most common change, followed by hyponatraemia (Peterson et al., 1996). Given the high occurrence of the alterations in sodium and potassium, the sodium and potassium ratio has historically been used to increase the clinical suspicion of hypoadrenocorticism. The normal sodium:potassium ratio ranges from 27:1 to 40:1 and lower ratios are thought to be more specific for cases of hypoadrenocorticism (Bartges and Nielson, 1992). A ratio of 24:1 has been suggested as a cut-off concentration for diagnosis of this condition, with a specificity of 100% (Adler et al., 2007). However, evidence for the use of sodium:potassium ratios in the diagnosis of hypoadrenocorticism is contradictory, with numerous other conditions resulting in hyperkalaemia and hyponatraemia (Adler et al., 2007, Nielsen et al., 2008, Roth and Tyler, 1999, Scott-Moncrieff, 2015, Schaer et al., 2001).

Conversely, some dogs with hypoadrenocorticism have normal electrolyte concentrations including dogs with atypical hypoadrenocorticism, as mineralocorticoid deficiency preventing renal electrolyte exchange is not a feature. Not all dogs with normal electrolytes have the atypical form of the disease however with some patients with hypoadrenocorticism demonstrating compensation for the aldosterone deficiency (Baumstark et al., 2014b). Either hyperkalaemia or hyponatraemia are more commonly associated with hypoadrenocorticism than changes in both electrolytes and up to 30% of patients have electrolyte concentrations completely within the normal range (Hughes et al., 2007). Therefore, it is currently considered important to measure aldosterone in patients with normal electrolytes, to distinguish between classical and atypical hypoadrenocorticism, however further research is needed in this area.

Other electrolyte abnormalities seen include hyperphosphataemia (68%), hypochloraemia (42%) and hypercalcaemia (32%) (Hess, 2018). These changes are probably due to altered electrolyte exchange within the kidneys due to mineralocorticoid deficiency (chloride) and glucocorticoid deficiency (calcium/phosphate) (Adamantos and Boag, 2008, Gow et al., 2009).

1.3.2.3 Biochemistry

Almost 90% of patients with hypoadrenocorticism are azotaemic which reflects the lack of reabsorption of water from the kidneys, hypotension and reduced renal perfusion. This can be marked and can be difficult to distinguish from an acute kidney injury (Peterson et al., 1996). Clinically, in hypoadrenocorticism there is often a quick resolution of the azotaemia with fluid therapy, however this is not the case with an acute kidney injury. Sometimes the increase in urea is proportionally more marked than the increase in creatinine. This can be in part due to gastrointestinal bleeding resulting in an increase in urea (Scott-Moncrieff, 2015).

Hypoglycaemia can occur in up to 30% of patients and in some cases can be severe enough to cause seizures (Lifton et al., 1996), particularly in dogs with atypical disease. This is because glucocorticoid deficiency causes reduced gluconeogenesis and peripheral sensitivity to insulin (Scott-Moncrieff, 2015). Another finding seen more commonly in dogs with atypical disease, but in all forms of hypoadrenocorticism, is hypoalbuminaemia (6-39%) and hypocholesterolaemia (7%). These changes are thought to be due to hepatic dysfunction (both albumin and cholesterol), protein losing enteropathy (albumin) and alterations in fat metabolism (cholesterol) (Scott-Moncrieff, 2015). As these patients often have increases in liver enzymes (30%), liver disease and hypoadrenocorticism can mimic each other both in terms of clinical signs and clinicopathological findings.

Finally, mineralocorticoid deficiency can result in a mild metabolic acidosis, due to impaired hydrogen ion excretion. It can also be due to hypovolaemia resulting in poor tissue perfusion and lactic acidosis (Kintzer and Peterson, 1997b).

1.3.2.4 Urinalysis

Although patients with hypoadrenocorticism are clinically dehydrated, they are unable to conserve water, and this results in a USG <1.030. Therefore, hypoadrenocorticism should be considered in dehydrated patients with a USG less than 1.030.

1.3.2.5 Imaging

Radiography, abdominal ultrasound and echocardiography are all useful in excluding differential diagnoses and significant co-morbidities or complications (Van Lanen and Sande, 2014, Whitley, 1995, Feldman and Peterson, 1984, Peterson et al., 1996, Melian et al., 1999). The use of imaging is discussed in more detail elsewhere (Spence et al., 2018).

1.4 Confirming the diagnosis of hypoadrenocorticism

1.4.1 Basal cortisol

A basal cortisol >55nmol/L has a sensitivity of 100% for the exclusion of hypoadrenocorticism (Bovens et al., 2014). This is more sensitive than the cut-off of 28 nmol/L which was previously used, which has a sensitivity of only 85.7% (Bovens et al., 2014, Lennon et al., 2007). Although basal cortisol is an excellent exclusion test, it cannot be used for diagnosis of hypoadrenocorticism. Basal cortisol concentrations <22 nmol/L have a specificity of 95.7-98.2% and when <55 nmol/L, a specificity of only 78% (Lennon et al., 2007, Gold et al., 2016). Cortisol concentrations <5.5nmol/L had a specificity of 99.1% (Gold et al., 2016), however it should be noted that the accuracy and precision of many cortisol assays at this concentration has not been tested.

Cortisol is stable in serum and urine at 4^{o}C and 25^{o}C for 5 days (Behrend et al., 1998). It is measured in plasma or serum by radioimmunoassay, chemiluminescent assay or enzyme-linked immunosorbent assay (Russell et al., 2007).

1.4.2 ACTH stimulation test

The standard diagnostic test for the diagnosis of hypoadrenocorticism is the ACTH stimulation test. The use and potential confounding factors of this test have been reviewed elsewhere (Scott-Moncrieff, 2015, Klein and Peterson, 2010b, Spence et al., 2018).

This test involves obtaining a serum sample for measurement of basal cortisol, then giving synthetic ACTH intravenously or intramuscularly and then taking a second serum sample for cortisol measurement 30 to 90 minutes later. A depot intramuscular injection with a longer acting formulation of ACTH containing inorganic zinc complexes has also been shown to be effective in the diagnosis of hypoadrenocorticism (Sieber-Ruckstuhl et al., 2015). The lowest dose of ACTH found to stimulate the adrenal gland is 0.5μg/kg (Martin et al., 2007) and the lowest dose found to cause maximal stimulation of the adrenal gland in patients suspected to have hypoadrenocorticism is 5 μg/kg (Lathan et al., 2008). There is no benefit of using higher doses of ACTH (such as 250μg/dog) and therefore 5 μg/kg has become the recommended dose (Lathan et al., 2008). No differences have been shown in peak cortisol concentration when comparing intravenously and intramuscularly administered ACTH (Cohen and Feldman, 2012). In addition, perivascular administration of synthetic ACTH has not been shown to affect ACTH stimulation test results in dogs with hyperadrenocorticism and therefore subcutaneous administration should not affect results of the ACTH stimulation test (Johnson et al., 2017). Most protocols suggest that post ACTH serum samples are collected at 60 minutes, however sampling time ranges from 30 to 90 minutes (Frank et al., 2000). Dogs do not exhibit circadian rhythm of cortisol release and therefore time of day is not an important factor in sampling (Scott-Moncrieff, 2015).

Diagnosis of hypoadrenocorticism is made when pre and post ACTH concentrations are <55 nmol/L and this has a sensitivity and specificity of 100% in diagnosing hypoadrenocorticism (in the absence of exogenous glucocorticoid administration). It does however not distinguish between dogs with spontaneous hypoadrenocorticism and those on exogenous glucocorticoid therapy. Most dogs with this condition have cortisol concentrations under 28 nmol/L before and after synthetic ACTH administration (Scott-Moncrieff, 2015).

1.4.3 Plasma ACTH concentrations

Plasma ACTH concentrations are increased in dogs with primary hypoadrenocorticism (Boretti et al., 2015) due to a lack of negative feedback of cortisol on the HPA axis. Plasma ACTH concentrations are also increased in patients with pituitary dependant hyperadrenocorticism, however the clinical presentation and ACTH stimulation test results would be very different between these two conditions (Scott-Moncrieff, 2015). ACTH to cortisol ratios (CAR) have been proposed as a promising screening test, however further studies are required (Boretti et al., 2015, Javadi et al., 2006, Lathan et al., 2014).

Plasma ACTH can be measured by a radioimmunoassay or a chemiluminescent assay, both of which have been validated in dogs (Scott-Moncrieff et al., 2003). Sample handling is very important and blood samples must be obtained in EDTA anti-coagulated blood and separated within 15 minutes of collection. Finally, the sample must be frozen immediately after separation (Scott-Moncrieff, 2015) and the sample should also not be in contact with glass at any point as ACTH adheres to glass, causing falsely low results.

1.4.4 Plasma aldosterone concentrations

In dogs with primary hypoadrenocorticism, plasma aldosterone tends to be below lower reference limits, however there is overlap between these patients and healthy dogs (Baumstark et al., 2014b). Many studies have looked at plasma aldosterone following ACTH stimulation however ACTH has only a minimal effect on plasma aldosterone at physiological levels. In patients with atypical hypoadrenocorticism, where there is glucocorticoid deficiency only, the plasma aldosterone concentrations are within the normal range (Thompson et al., 2007, Lifton et al., 1996).

Plasma aldosterone is measured by a radioimmunoassay (Sieber-Ruckstuhl et al., 2006). The test is run on serum samples and as with cortisol, plasma aldosterone is relatively stable. Samples can be stored for 7 days between 2C and 8C and for up to two months at temperatures less than -20C (Scott-Moncrieff, 2015).

1.4.5 Plasma renin activity

When aldosterone is low, plasma renin activity increases (Montori and Young, 2002). Therefore, dogs with classical primary hypoadrenocorticism have an increase in plasma renin activity (Baumstark et al., 2014a). Plasma renin activity is measured using an angiotensin radioimmunoassay (Boer et al., 1985). As with plasma ACTH measurement, samples should be obtained in EDTA anti-coagulated blood and separated within 15 minutes of collection before freezing.

1.4.6 Aldosterone-to-renin ratio

With both plasma aldosterone and plasma renin activity, there can be overlap between healthy dogs and dogs with hypoadrenocorticism. The aldosterone to renin ratio is a much more sensitive test of mineralocorticoid deficiency and classical hypoadrenocorticism (Javadi et al., 2006), with no observed overlap between these two groups.

1.5 Treatment of hypoadrenocorticism

Treatment of hypoadrenocorticism is largely dependent on whether patients present as an emergency in an acute adrenal crisis or whether they have more chronic, less severe signs. Treatment also depends on whether a diagnosis has been made, as many treatments can interfere with diagnostic confirmation of the condition as discussed (Spence et al., 2018).

1.5.1 Acute management

1.5.1.1 Intravenous fluid therapy

Most patients who present acutely have a severe mineralocorticoid deficiency, resulting in dehydration, hypovolaemia and hypotension, often with concurrent electrolyte disturbances. Therefore, aggressive fluid therapy is the mainstay of treatment in the acute setting, and appropriate fluid therapy can alleviate all signs in the short term, whilst awaiting results of diagnostic testing.

Crystalloid fluid therapy is the most appropriate choice and more specifically, 0.9% sodium chloride provides the most rapid correction of the hyponatraemia and hypokalaemia. It is however less effective compared to compound sodium lactate at resolving metabolic acidosis when present. When patients have a marked hyponatraemia (<120mmol/L), 0.9% sodium chloride should be avoided as rapid correction of hyponatraemia can lead to delayed pontine osmotic demyelination (O'Brien et al., 1994, Churcher et al., 1999). This results in delayed and severe neurological deficits and is associated with correction of sodium at rates quicker than 10-12mmol/L per day of 0.5mmol/L per hour (Churcher et al., 1999). If there are concerns over marked hyponatraemia or rapid increase in sodium concentrations then compound sodium lactate would be the preferred choice of fluid. Colloid fluid therapy is rarely required unless there is a marked hypoalbuminaemia which could be exacerbated by aggressive fluid therapy (Scott-Moncrieff, 2015).

An adrenal crisis is one of the few situations where true shock rates of intravenous fluid therapy are required. This can be achieved by giving repeated boluses of 10 to 20 ml/kg over 15 minutes with frequent reassessment during this period to address the patient's response and requirement for on going aggressive fluid therapy. Often rates of 40 to 80ml/kg are required over the first two hours (Gunn et al., 2016).

1.5.1.2 Management of electrolyte disturbances

In most patients, initiation of fluid therapy rapidly corrects electrolyte disturbances or at least reduces potassium concentrations from dangerous to safe concentrations. If hyperkalaemia does not resolve within the first 15 minutes of treatment, if there is a persistently marked hyperkalaemia of >7.0mmol/L or if there is a severe bradycardia, then treatment can be considered (Scott-Moncrieff, 2015). Intravenous boluses of 10% calcium lactate (0.5ml/kg) diluted and given over twenty minutes will not reduce the potassium concentrations but will be cardioprotective (DiBartola, 2001), increasing the heart rate whilst the plasma serum potassium concentrations reduce with on going fluid therapy (Scott-Moncrieff, 2015). If potassium concentrations do not decrease with fluid therapy, then insulin (0.2 IU/kg) and glucose (1 – 2g/unit of insulin) can be administered (Scott-Moncrieff, 2015). This therapy drives insulin intracellularly however potassium, glucose and phosphate (which is also driven intracellularly) should all be monitored closely.

1.5.1.3 Acute glucocorticoid and mineralocorticoid supplementation

Ideally an ACTH stimulation test should be performed before giving any exogenous steroid preparation. If a patient is haemodynamically unstable before completion of an ACTH stimulation test, then dexamethasone is the steroid of choice to administer as it does not cross react with commercial cortisol assays. It can however affect the HPA axis by causing negative feedback to the pituitary gland, reducing ACTH release and therefore cortisol release. This means that the ACTH stimulation should be completed as soon after administering dexamethasone as possible (Kemppainen et al., 1989). It is important to note that dexamethasone is a pure glucocorticoid and will have no effect on mineralocorticoid deficiency. Hydrocortisone does however have equal mineralocorticoid and glucocorticoid effects and can be given as mineralocorticoid supplementation. As it does cross react with the cortisol assay, it should not be administered until post ACTH stimulation serum samples are obtained. Generally, steroid doses administered during adrenal crisis are variable but are most often increased to three to five times the physiological dose. This results in doses of 0.1 to 0.2 mg/kg of dexamethasone intravenously or 5mg/kg of hydrocortisone sodium succinate intravenously (Scott-Moncrieff, 2015). It has also been shown that a constant rate infusion of hydrocortisone sodium succinate (0.5 – 0.625mg/kg/hour) results in a more rapid normalisation of electrolytes and clinical resolution of signs (Gunn et al., 2016).

1.5.2 Chronic management

Glucocorticoid supplementation is required in all dogs with hypoadrenocorticism, whether they have the classical or atypical form of the disease (Klein and Peterson, 2010b, Spence et al., 2018). Mineralocorticoid supplementation is only required when there are electrolyte derangements or inappropriately low aldosterone (Baumstark et al., 2014b, Wakayama et al., 2017).

1.5.2.1 Prednisolone

In stable patients, the most commonly used formulation for glucocorticoid supplementation is prednisolone tablets given once a day. Prednisolone is primarily a glucocorticoid and does not provide sufficient mineralocorticoid supplementation(Hess, 2018). Side effects of this drug include polyphagia, polydipsia, polyuria, excessive panting and coat thinning (Plumb, 2018).

The starting dose of prednisolone is 0.1 – 0.22mg/kg/day, however a wide range of doses from <0.05mg/kg to 0.4mg/kg per day have been reported (Kintzer and Peterson, 1997b). During times of physiological stress such as elective surgery or concurrent illness, glucocorticoid requirements may increase. Therefore, doses of glucocorticoid may need to be increased up to three to 5-fold (Klein and Peterson, 2010b).

1.5.2.2 Fludrocortisone

Fludrocortisone acetate is another formulation of steroid with both glucocorticoid and mineralocorticoid activity. Therefore, prednisolone is often not required as an adjunctive therapy long term. It has however been shown that the use of prednisolone in the initial management decreases the time until clinical stabilisation of the condition (Roberts et al., 2016).

There are no licensed preparations of fludrocortisone for use in dogs. Early studies showed the range of fludrocortisone dose given subcutaneously in adrenalectomised dogs to be 0.38-3.6 μg/kg/day (Parlow et al., 1956). The current starting dose is 0.02 mg/kg/day, split over one or two doses (Plumb, 2018). The mean dose in stable patients is 0.01 to 0.08mg/kg/day orally (Scott-Moncrieff, 2015, Kintzer and Peterson, 1997b, Roberts et al., 2016). Although historically it was recommended to supplement this therapy with salt administration, this has not been shown to improve outcome (Kintzer and Peterson, 1997b).

1.5.2.3 DOCP

Desoxycorticosterone pivalate (DOCP) is a pure mineralocorticoid used in the chronic management of hypoadrenocorticism (McCabe et al., 1995, Lynn and Feldman, 1991, Lynn et al., 1993, Van Zyl and Hyman, 1994). As aldosterone can have a minimal effect on glucocorticoid receptors (Scott-Moncrieff, 2015), it is possible that it can result in very mild glucocorticoid activity. DOCP is a long-acting version of deoxycortisterone acetate (DOCA) which was initially formulated in the 1940's and was investigated clinically in the 1950's in adrenalectomised dogs (Baker et al., 1954). There was unequivocal evidence that a solubilised formulation of the drug was safer than a suspension which had resulted in a high rate of anaphylactic shock in rats. Of this solubilised formulation, the starting dose was found to be 125-250 μg/kg/day.

There are currently two microcrystalline licensed preparations available for use in dogs: Percorten® (Novartis) which is licensed in North America and Zycortal® (Dechra Ltd) which is licensed for use in dogs in Europe and North America. Both are available in a 25mg/ml prolonged-release formulation and Zycortal® has been shown to be non-inferior to Percorten® (Farr et al., 2020). DOCP has a rapid onset of action and therefore only a brief transition period of around 48 hours is required when transitioning from parenteral steroids (hydrocortisone) or fludrocortisone acetate (Scott-Moncrieff, 2015, Ramsey et al., 2016).

The authorised starting dose of the currently used formulations of DOCP is 2.2mg/kg given subcutaneously (or intramuscularly in the case of Percorten®) every 25 – 28 days. Often a much lower dose is required for long term management, with the Percorten® dose range being 0.8 – 3.4mg/kg dose every 14 – 35 days (Kintzer and Peterson, 1997b). Peak action of this drug is most common around day 10 (Jaffey et al., 2017). Side effects of overdose or under dose include hypotension and polydipsia (due to the hypernatraemia/hypokalaemia), depression, lethargy and gastrointestinal signs (Plumb, 2018). Lower doses of 1.0mg/kg have been shown to provide effective mineralocorticoid supplementation in some dogs with no adverse effects (Bates et al., 2013). However it is difficult to predict which dogs need more or less mineralocorticoid supplementation and therefore higher starting doses are required before tapering the drug to lower doses (Scott-Moncrieff, 2015).

Electrolyte concentrations 10-14 days following an injection reflect the peak activity of the drug and provide information on the drug dose used (Mason et al., 2017). Electrolyte concentrations at day 25-28 following injection provide information on the duration of action of the drug (Ettinger et al., 2017, Lynn et al., 1993, Hess, 2018). The dosing interval has been extended in one study with a dosing frequency ranging from 30-90 days with a 54-60% reduction in cost of treatment (Jaffey et al., 2017). In this study, 10% of dogs exhibited clinical signs of hypoadrenocorticism or electrolytes outwith the reference range. Extending the dosing interval did not result in an Addisonian crisis in any of these patients. DOCP is 90% bound to plasma proteins with only 10% free to exert a biological effect (Drugbank, 2019). This could result in prolonged duration of action of DOCP and is speculated to be the reason why individual dosing interval is variable between patients, however further studies are required (Jaffey et al., 2017) to investigate this. Marked variation has been noted between individuals since the earliest of studies (Baker et al., 1954) and it is for this reason that some clinicians believe that dosing intervals should not be extended as this can lead to an acute adrenal crisis.

1.6 Monitoring of patients with hypoadrenocorticism

1.6.1 Clinical signs

Clinical signs are very important in assessing the clinical control of hypoadrenocorticism, regardless of which therapy patients are being treated with. It is particularly important in the case of assessing glucocorticoid supplementation, as there are no readily available, routine diagnostic tests available. As prednisolone and fludrocortisone both cross-react with cortisol assays (Krasowski et al., 2014) but do not give biologically equivalent results to endogenous cortisol, basal cortisol and ACTH stimulation tests serve no purpose following the initial diagnosis of hypoadrenocorticism. Although increases in alkaline phosphatase and a stress leukogram on haematology are often present with excessive steroid therapy (Moore et al., 1992), these are inaccurate markers of glucocorticoid supplementation.

Monitoring glucocorticoid supplementation involves decreasing the prednisolone dose when clinical signs of steroid overdose are present (polyuria, polydipsia, polyphagia, excessive panting and coat thinning) and increasing the dose when patients show signs of glucocorticoid deficiency (lethargy and gastrointestinal signs) (Scott-Moncrieff, 2015, Lathan and Thompson, 2018, Farr et al., 2020). Therefore, a thorough history is required at re-check visits.

Unfortunately, there can be some overlap between clinical signs of glucocorticoid excess, glucocorticoid deficiency, mineralocorticoid excess and mineralocorticoid deficiency. For example, lethargy and weakness can indicate either glucocorticoid deficiency or mineralocorticoid deficiency and occasionally, mineralocorticoid excess.

1.6.2 Electrolytes

Drugs such as DOCP and fludrocortisone acetate reduce potassium concentrations and increase sodium concentrations. Therefore, hypernatraemia and hypokalaemia are markers of mineralocorticoid excess and these are most commonly seen around day 10 following an injection of DOCP around the time of peak action or at any point during treatment with fludrocortisone acetate. In contrast, hyperkalaemia or hyponatraemia indicate mineralocorticoid deficiency.

The aim throughout therapy is to maintain sodium and potassium concentrations within their respective reference ranges, ideally within the middle third of the range (Lathan and Thompson, 2018, Adler et al., 2007).

Sodium:potassium ratios have been used to both diagnose hypoadrenocorticism but also to monitor therapy. A sodium:potassium ratio of 27-32 has been suggested by the manufacturers of Zycortal® (Farr et al., 2020) to be an appropriate target range from patients receiving this drug. This is based on studies which show 27 to be a sensitive marker for mineralcorticoid deficiency (Adler et al., 2007, Scott-Moncrieff, 2015, Nielsen et al., 2008, Roth and Tyler, 1999). The upper limit of the "normal" ratio range is 40 (Scott-Moncrieff, 2015), meaning 32 is an arbitrary number and there is no evidence suggesting that dogs with sodium:potassium ratios <32 are clinically better controlled than those with higher rations.

1.6.3 Plasma ACTH concentrations

Although plasma endogenous ACTH concentrations have been shown to be high in patients with untreated hypoadrenocorticism (Zeugswetter and Schwendenwein, 2014, Lathan et al., 2014), it is unknown if endogenous ACTH concentrations are of benefit in assessing clinical control in patients being treated for hypoadrenocorticism. Studies in human medicine show that exogenous glucocorticoid therapy results in HPA suppression (Russell et al., 2010, Paragliola et al., 2017) and therefore endogenous ACTH concentrations could be of clinical utility.

1.6.4 Plasma renin activity

Plasma renin activity provides additional information on mineralocorticoid control (Baumstark et al., 2014a). Plasma renin activity reflects mineralocorticoid supplementation as patients with untreated hypoadrenocorticism have hypovolaemia and reduced glomerular filtration rate (Scott-Moncrieff, 2015). This results in an increase in renin activity. It is unknown if patients with excessive mineralocorticoid supplementation have undetectably low renin activity due to a negative feedback response.

1.6.5 Blood pressure

Blood pressure has been studied in 8 dogs on treatment with DOCP for management of hypoadrenocorticism (Kaplan and Peterson, 1995). None of the dogs developed systolic or diastolic arterial hypertension during the 105 day study period (measured on days 0, 30, 60, 75, 90, and 105). Patients with mineralocorticoid over-supplementation (particularly DOCP overdose) are expected to be hypernatraemic and this leads to excessive water retention in the kidneys. None of the dogs in the Kaplan and Peterson study were considered to be over supplemented with DOCP at a standard dose of 2.2mg/kg every 30 days and therefore it is unknown whether or not blood pressure is affected by overdose of this drug.

1.7 Prognosis

In dogs with naturally occurring autoimmune destruction of their adrenal glands, the long term prognosis is good, depending on timely diagnosis and management of the condition. Age at diagnosis, breed and sex did not affect prognosis and the median survival time of dogs with hypoadrenocorticism in one study was 4.7 years (Kintzer and Peterson, 1997b).

1.8 Study design

Effectiveness studies are used to compare the efficacy of two different drugs and include superiority trials, equivalence trials and non-inferiority trials. Although in most circumstances it would be preferable to show superiority of one drug compared to another, this is often not practically possible as the numbers needed from superiority power calculations are not attainable, particularly in veterinary medicine. Therefore one drug instead can be shown to be non-inferior to another, meaning its success is not clinically significantly worse than other (Evans, 2010). In addition, many drugs have practical benefits over another such as reduced dosing frequency or expense and often the main aim is to show that a test drug is "no worse than" a control drug, rather than being more effective (Schumi and Wittes, 2011).

One of the key steps in designing a non-inferiority study is deciding the non-inferiority margin (effectiveness margin or delta). There are numerous methods for this, however in around 60% of published human medical non-inferiority trials, the method is not defined which is a failing of these studies (Althunian et al., 2017). In a recent review of the human literature, 36.5% of studies that did define how the non-inferiority margin was calculated, reported that the decision was based upon expert clinical opinion (Althunian et al., 2017). This means that rather than a formal statistical approach, experts instead decide upon a percentage difference in success between two drugs (the active control and the test drug), which is considered clinically acceptable. This percentage difference takes into consideration various factors including how efficacious the control drug is, how much efficacy needs to be retained and the importance of benefits the test drugs may have. For example, if a control drug has a large impact on a patient's quality of life (ie the effect of the control drug compared to placebo is large), the percentage difference between the test drug and the control drug must be small. If a control drug has a smaller impact on quality of life and the patient's health is less reliant on this drug (ie there is less difference in effect between the control drug compared to placebo), then a larger reduction in efficacy of the test drug compared to the control drug may be accepted in exchange for test drug benefits such as increased palatability or reduced dosing frequency (Food and Drug Administation, 2016).

The approach of defining the non-inferiority margin based on expert clinical opinion is common in veterinary medicine. This is as a statistical approach to defining the margin relies on analysis of the outcome of previous studies and often the literature is not sufficient for reliable statistical analysis. In 2010, the US Food and Drug Administration (FDA) drafted guidelines (later revised in 2016) on how to define the margin based on clinical and statistical considerations. These guidelines describe the fixed margin approach (also known and to 95%-95% method) and the synthesis approach and these methods should be used where possible (Food and Drug Administation, 2016).

The non-inferiority margin must be set before sample size is considered, as non-inferiority power calculations require: the significance level (alpha), the power level (1-beta), the success of the control drug in previous studies, the success of the test drug in previous studies and the non-inferiority limit (Food and Drug Administration, 2015). For interpreting the non-inferiority results, set criteria must be established to define success and these same criteria apply for both groups. The 95% confidence interval of the difference between the successes of the two drugs is then calculated. For non-inferiority, the lower bound of this confidence interval must be within the non-inferiority margin (Oczkowski, 2014).

1.9 Aims

The aim of this study was to compare the efficacy of fludrocortisone acetate and DOCP in the management of canine patients with spontaneous hypoadrenocorticism. Although both treatments have been shown to be successful in controlling clinical signs of the condition (Lynn et al., 1993, Lynn and Feldman, 1991, Lathan and Thompson, 2018, Lathan et al., 2008), the two drugs have never been directly compared. Instead, dogs have been switched from one drug to another following treatment failure (Van Zyl and Hyman, 1994).

2 Materials & Methods

2.1 Hypothesis

The hypothesis of this study was that treatment with a 25mg/ml prolonged-release suspension for injection of DOCP (the interventional product) with prednisolone, was not inferior to treatment with fludrocortisone acetate 0.1mg tablets (the control product) in the management of canine hypoadrenocorticism.

The null hypothesis was that the treatment success (percentage of dogs with normal concentrations of sodium and potassium and no clinical signs) at each observation point on fludrocortisone, is 22% or more than the percentage reported as on DOCP with prednisolone.

The alternate hypothesis was that the treatment success (percentage of dogs reported as having normal concentrations of sodium and potassium or having clinical signs) at each observation point on DOCP with prednisolone is less than 22% of the percentage reported when on fludrocortisone.

2.2 Study design

The study was carried out at the Small Animal Hospital, University of Glasgow, between January 2016 and January 2017. This study was a single-site, randomised, non-masked, cross-over design study (Freise et al., 2013, Huitfeldt et al., 2011). Masking was not possible as the DOCP and fludrocortisone are given by different routes and the fludrocortisone requires daily dosing at home. A placebo arm of the trial was also not possible as withholding treatment in hypoadrenocorticism can lead to fatal decompensation. Ethical approval was granted by the University of Glasgow Ethics Committee and the study was conducted following the receipt of an Animal Test Certificate (Type B) [ATC-B] from the Veterinary Medicine Directorate.

2.2.1 Determination of sample size

Power calculations were performed using an online randomisation tool (www.sealedenvelope.com) to assess the number of dogs required to be recruited to show non-inferiority of the investigational product compared to the control product, based on previous studies on the efficacy of DOCP (Kintzer and Peterson, 1997b, Melian and Peterson, 1996, Farr et al., 2020). To show superiority, an impractical number of dogs would be required to be enrolled as canine hypoadrenocorticism is an uncommon condition. Therefore it was decided that a non-inferiority study would be more appropriate and would still address the hypothesis.

The expected success of the DOCP was 86% (Farr et al., 2020) and for the fludrocortisone was 84% (Melian and Peterson, 1996, Kintzer and Peterson, 1997b). Alpha (the significance level) was set at 5% and the non-inferiority margin was set at 22%, as the lead investigator felt that a difference in success of this level would be clinically significant, meaning that the investigational product would result inadequate management of hypoadrenocorticism. It was found that thirty dogs in a crossover design would provide more than 80% power to investigate the hypothesis. This means that if the success of the experimental product was more than 22% less than the control product, the experimental product would be considered non-inferior.

2.2.2 Treatment Groups

There were two treatment groups (A and B) with a cross over at the midpoint of the study, following a transition (washout) phase. Group A dogs began the trial on DOCP and concluded the trial on fludrocortisone. Group B dogs began the trial on fludrocortisone before starting DOCP following the crossover. Fludrocortisone was given to all patients during the transition period. Patients were randomly assigned at the point of entry to each group using a computer generator, at a ratio of 1:1.

2.2.3 Study and visit schedule

The dogs were started on treatment with either DOCP and prednisolone (Group A) or fludrocortisone (Group B) and continued this treatment for three months, before embarking on a 28 day washout period (during which time they were treated with fludrocortisone). They then received three months of the alternative treatment. Figure 1 illustrates the study design.

Figure 1. Diagrammatic representation of the Study Design

Figure 1a. Original Study Design

The treatment course of dogs allocated to groups A and B before Florinef® came off the market. This shows the 4 week washout period during the cross over for dogs in both groups, meaning dogs in group B being treated with fludrocortisone stayed on this medication for an extra month before receiving their first injection of DOCP. Therefore, the length of the trial was the same for all dogs, regardless of which group they were in.

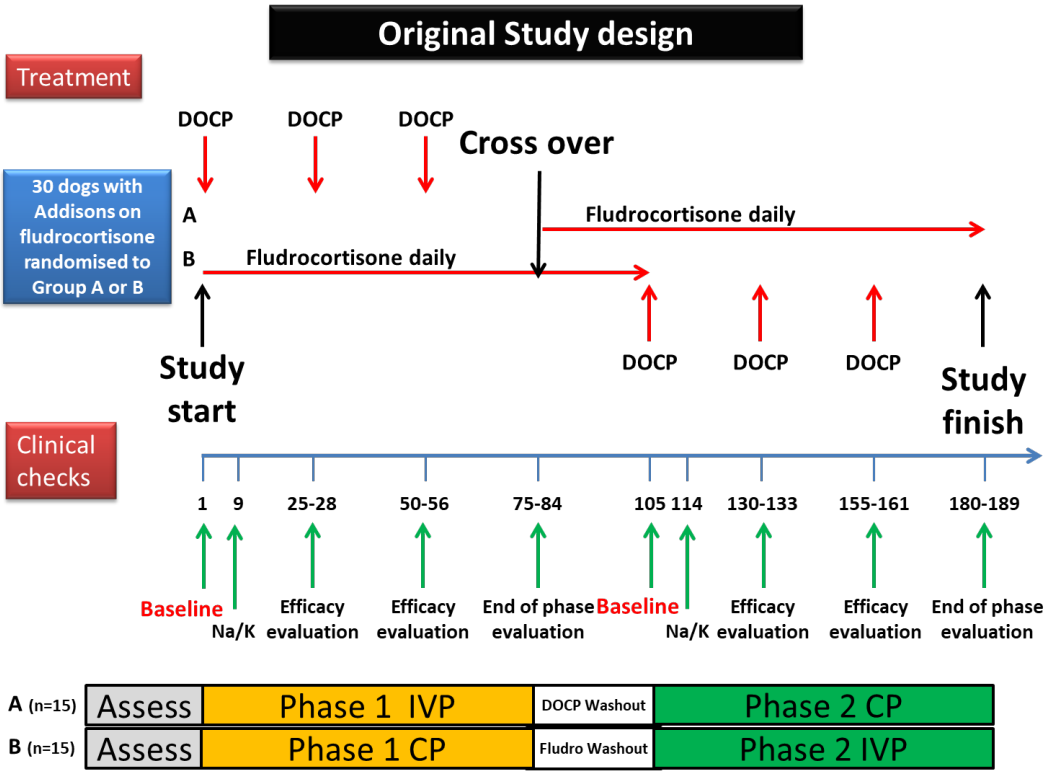
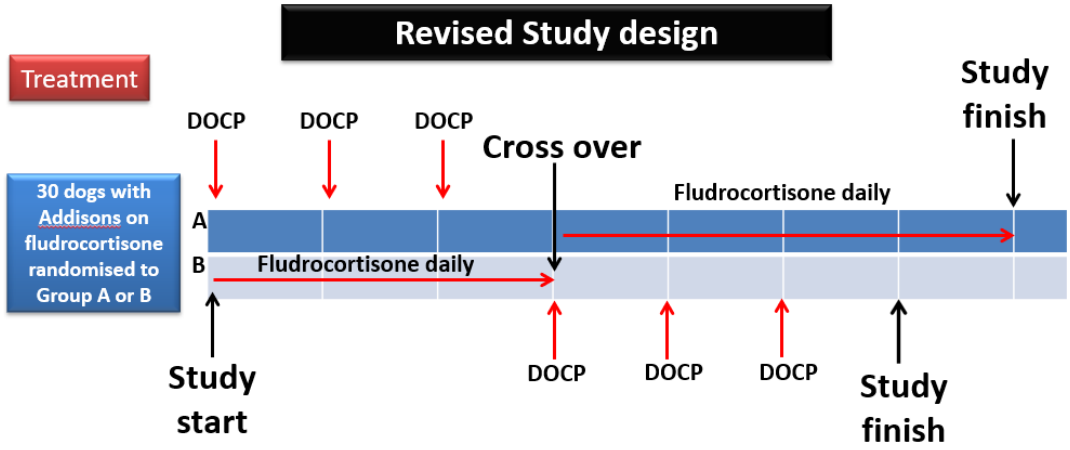
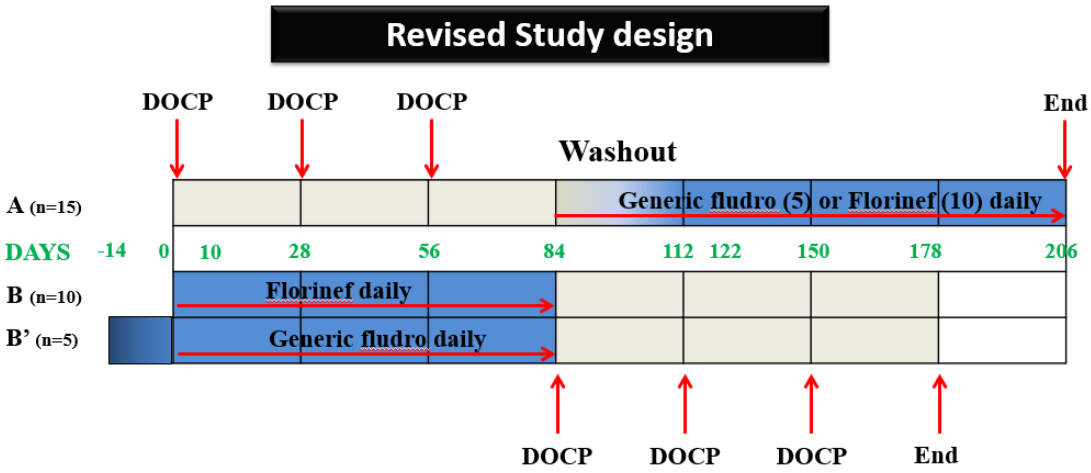


Figure 1b. Design after Florinef® became unavailable

The study design after Amendment 2, which was an adjustment made to the Study Protocol after Florinef® came off the market. Following this, generic fludrocortisone was used which was significantly more expensive. Therefore the 4 week washout period was lost in group B dogs which were treated with fludrocortisone, as this was not required. The washout period in these dogs was only to keep the trial length equal in all dogs. The dogs in group B finished the trial four weeks earlier than those in group A.





At the initial consultation, a full clinical examination was performed and echocardiography, blood pressure, haematology, biochemistry, T4/TSH, ACTH and renin samples were all submitted. Ten days after the start of each phase, a full clinical examination, blood pressure and electrolytes were checked. Patients were checked every 28 days from the first visit. A full check as per the initial visit was performed at the end and start of each phase. Table 1 and Table 2 on summarise which tests were carried out at each visit.

Table 1. Study Schedule

This study schedule illustrates each phase of the trial, when each visit was expected to take place, tests run at each phase and the treatment given, depending on the group allocation.

	Visit	Study day	Owner interview, clinical exam, blood pressure, Na^+, K^+	Haematology, biochemistry, ACTH, renin	Group A Treatment: Phase 1: DOCP ‡ Phase 2: Fludrocortisone	Group B Treatment: Phase 1: Fludrocortisone Phase 2: DOCP ‡
	A0 *	0-13	X †			Fludrocortisone administered twice daily until the last dose on the day of Visit E1
Treatment	A1	1	X †	X	DOCP administered	
	B1	8–10	X			
	C1	28	X		DOCP administered	
	D1	56	X		DOCP administered	
	E1/A2	84	X	X	Fludrocortisone started	DOCP administered
Treatment phase 2	B2	93	X		Fludrocortisone administered twice daily; last dose on the day of Visit E2	
	C2	109–112	X			DOCP administered
	D2	140	X			DOCP administered
	E2	168	X	X		

*Only for dogs assigned to Group B.
† Included completion of Owner informed consent agreement on Day -13 for dogs in Group B and Day 1 for dogs in Group A.
‡ During treatment with DOCP, prednisolone was administered daily.

Table 2. Summary of data collected at each visit

This table summarises the samples collected at each visit and whether the results were used as a primary outcome measure (primary parameter) or secondary outcome measure (secondary parameter).

Parameter	Procedure	Time point	Statistical analysis
Sodium	Heparinised plasma sample	A1, B1, C1, D1, E1, A2, B2, C2, D2, E2	Primary parameter
Potassium	Heparinised plasma sample	A1, B1, C1, D1, E1, A2, B2, C2, D2, E2	Primary parameter
Na^+ / K^+ratio	Manual calculation	A1, B1, C1, D1, E1, A2, B2, C2, D2, E2	Primary parameter
Clinical signs	Physical examination, clinical history	A1, B1, C1, D1, E1, A2, B2, C2, D2, E2	Primary parameter
ACTH	Frozen EDTA sample	A1, E1, A2, E2	Secondary parameter
Renin	Frozen EDTA sample	A1, E1, A2, E2	Secondary parameter
Blood pressure	Tail base reading; oscillometric reading	A1, B1, C1, D1, E1, A2, B2, C2, D2, E2	Secondary parameter
Haematology	EDTA whole blood sample (not frozen)	A1, E1, A2, E2	Secondary parameter
Biochemistry	Heparinised plasma sample	A1, E1, A2, E2	Secondary parameter

2.3 Case selection

All dogs were actively recruited from first opinion practices. Practices in the South West of Scotland were contacted and asked to recruit patients being treated with fludrocortisone for treatment of hypoadrenocorticism. Permission was obtained to contact the owners directly to discuss the study protocol and to request consideration of patient enrolment. Before enrolment, case histories were thoroughly checked to ensure compliance with the inclusion criteria. Informed consent was obtained from all owners before patient enrolment on the study. In particular, this emphasised the risks involved in transitioning patients on to a new medication and the requirement for increased vigilance and record keeping.

2.3.1 Inclusion criteria

For inclusion, dogs had to have been diagnosed at least 60 days before enrolment on the trial. Diagnosis had to have been made following documentation of a lack of response to synthetic ACTH during an ACTH stimulation test, with cortisol samples submitted to a reference laboratory. They also had to be on twice daily treatment with fludrocortisone (at the same dose for at least 30 days before enrolment), with electrolytes within their respective reference ranges at the time of the first visit. Dogs were not excluded if they were on prednisolone therapy at the time of enrolment. Dogs also had to be amenable to blood sampling and travelling to the study site regularly. Finally, they had to be a minimum of 5kg in weight given the volume of blood sampled at each visit.

2.3.2 Exclusion criteria

Dogs were excluded if they were enrolled in any other studies using an investigational product, if they were pregnant or lactating, had signs of primary hepatic failure, signs of renal insufficiency (azotaemia and isothenuria), diagnosed with congestive heart failure, on any other immunosuppressive therapy (eg for skin disease or other autoimmune conditions) or on any other steroid preparations (excluding oestriol for management of urinary incontinence) other than that prescribed for use in the study. Patients with concurrent diabetes mellitus, hypothyroidism or urinary incontinence were not excluded provided patients were considered stable on treatment of these conditions and only one of these conditions had been diagnosed.

2.3.3 Recording of data and study monitoring

Each case was assigned a folder containing a visit schedule and the relevant forms to be completed at each visit. A full clinical examination was performed and recorded, along with blood pressure, at each visit. All treatments were recorded at each visit, as well as a questionnaire regarding drug side effects. Each page of the folder was signed and dated by the veterinarian conducting the check. At each visit, the owner also completed a quality of life questionnaire. During and at completion of a trial, all the data were independently checked for errors and omissions.

2.4 Treatment

2.4.1 Control product

Fludrocortisone acetate 0.1mg tablets were used in this study. These were either a generic formulation (Aspen) or Florinef® (Bristol Squibb Myers). The latter product required refrigeration however the former did not. Neither of these drugs are licensed for use in dogs for the treatment of hypoadrenocorticism. These medications were given orally at home by the owner, twice a day. Owners were asked to record each time the medication was administered to confirm adherence to the study protocol. If on only once daily administration before study recruitment, patients were transitioned onto twice daily dosing for at least two weeks before starting the trial. The dose used was pre-determined by each patient's individual requirements, depending on the dose which resulted in electrolyte concentrations within the normal range and the absence of clinical signs of hypoadrenocorticism. Doses were unchanged during the study, unless patients developed electrolyte concentrations out with their respective normal reference ranges.

2.4.2 Interventional product

A 25mg/ml prolonged release suspension for injection of desoxycorticosterone pivalate (DOCP, Zycortal®, Dechra Ltd.) was used. This is a licensed drug for the management of hypoadrenocorticism in canine patients. Handling considerations include gentle inversion of the bottle to re-suspend the product before injection. The DOCP was stored at room temperature at the study site.

The DOCP was administered every 28 days by subcutaneous injection, by one of four experienced vets. The initial starting dose was 2.2mg/kg in all patients, with doses being tailored to each patient's individual requirements during the study, based on electrolyte concentrations at day 10 post-injection and clinical signs.

As DOCP is a pure mineralocorticoid, glucocorticoid supplementation was also required with the DOCP. Prednisolone tablets (1mg, 5mg and 25mg) were administered once daily by the owner at home. Owners were asked to record each time the medication was administered to confirm adherence to the study protocol. The starting dose was 0.2mg/kg orally, once a day. Doses were adjusted based on clinical signs of glucocorticoid excess (polyuria, polydipsia, polyphagia, panting and coat growth) and deficiency (lethargy and gastrointestinal signs). The dose was increased by up to 100% during times of stress during the study.

2.4.3 Dose adjustments

Either a European Specialist in Small Animal Internal Medicine or a Resident in Small Animal Internal Medicine made dose adjustments. Owners were strictly prohibited from making dose adjustments of prednisolone at home, regardless of drug-related side effects. Prednisolone dose adjustments were made purely on the presence of clinical signs of glucocorticoid over or under dose. Hyperkalaemia or hyponatraemia would prompt dose increases and hypokalaemia or hypernatraemia would prompt dose reductions in DOCP. Table number 3 was used to assist with DOCP dose adjustments.

Table 3. DOCP dose adjustments; original plan

This table shows the original plan for making dose DOCP adjustments, prior to the study amendment, which allowed larger dose reductions.

Na:K Ratio	Dose Adjustment
>34	Decrease dose to 2.0mg/kg
32 to <34	Decrease dose to 2.1mg/kg
27 to <32	Continue 2.2mg/kg
24 to <27	Increase dose to 2.3mg/kg
<24	Increase dose to 2.4mg/kg

2.4.4 Concurrent Medications

Concurrent medications for management of conditions other than hypoadrenocorticism were not excluded during the study period. However, drugs which could interfere with the study objectives were prohibited. These included potassium depleting diuretics which could result in hypokalaemia and any steroid preparations other than those prescribed for use in the study.

2.5 Monitoring

2.5.1 Blood pressure

Blood pressure was measured at each visit using a Cardell Veterinary Monitor (Model 9401). This is a validated oscillometric method of blood pressure measurement (Mitchell et al., 2010, Bosiack et al., 2010, Sawyer et al., 2004). The cuff was placed at the tail base with patients standing. Cuff size was recorded to ensure consistency in sampling and an average was taken of five readings for analysis.

2.5.2 Blood sampling

Jugular blood samples were taken from all patients. Where possible, 10 millilitres of blood was obtained to ensure adequate volumes for the necessary tests. Any residual blood was stored at -20^{o}C and later -80^{o}C for future sampling if required, for one year after trial completion. A portion of residual EDTA anti-coagulated blood was separated before freezing, to allow measurement of plasma ACTH and plasma renin activity.

2.5.2.1 Routine haematology and biochemistry

Samples for routine haematology and biochemistry were obtained in EDTA and lithium heparin tubes respectively. These were submitted to the Veterinary Diagnostic Services laboratory, Glasgow, for reference haematological and biochemical results (ADIVA 120 Haematology Analyser, Siemens and AU640 Series Analyser, Olympus). Samples were run within 48 hours of collection and were stored at 4^{o}C if samples were not being processed immediately.

2.5.2.2 Electrolytes

The electrolytes were measured using an in-house analyser (Catalyst Dx, IDEXX One IDEXX Drive, Westbrook, Maine 04092) and on the basis of these results the doses of DOCP or fludrocortisone were adjusted as necessary. In addition, the electrolytes were also measured within 48 hours using a commercial laboratory analyser (Olympus AU640 series or Siemens Dimension Xpand Plus).

2.5.2.3 ACTH concentrations

Blood was collected in EDTA anti-coagulated tubes which were separated within 15 minutes, with the plasma transferred into a plain tube before storage at -80^{o}C. Samples were labelled and shipped to Nationwide Specialist Laboratories, Cambridge, in batches. The maximum storage time was six months. ACTH was measured using a competitive enzyme linked immunoassay (Canine ACTH ELISA, Biomerica Inc, 17571 Von Karman Avenue, Irvine, CA 92614, USA. Product Ref: 7023) (Scott-Moncrieff et al., 2003).

2.5.2.4 Plasma renin activity

Samples were handled as for plasma ACTH measurement. Renin was measured using a previously validated plasma renin activity radioimmunoassay (GammaCoat, Plasma Renin Activity Kit, DiaSorin Via Crescentino SNC, 13040 Saluggia, Italy. Product Ref: CA1533) (Locsei et al., 2009, Martinez et al., 2017). Each sample was measured in duplicate and the mean of the results used. Where the co-efficient of variation between the two values was greater than 15%, the test was repeated.

2.6 Outcome measures

2.6.1 Primary Outcome Measures

Effectiveness was assessed primarily based on sodium and potassium concentrations when measured every four weeks throughout the study period. These were used to assess the efficacy of mineralocorticoid supplementation. The frequency of clinical signs of glucocorticoid excess (polyuria, polydipsia, polyphagia, panting and poor coat re-growth) and deficiency (lethargy and gastrointestinal signs) were used to assess glucocorticoid supplementation.

2.6.2 Secondary outcome measures

Additional assessment of outcome was made by endocrine (ACTH and plasma renin activity) and blood pressure assessment. Haematology and biochemistry results were analysed to provide supplementary information however were not used as primary outcome measures.

2.7 Statistical analysis

2.7.1 Data analysis

All data was recorded on an Excel spreadsheet. Statistical analysis was performed using a statistical software package (Minitab 19.2, Minitab LLC, USA). The Anderson-Darling test was used to assess for normality for each variable at each time point assessed. Paired T-tests were used when comparing normally distributed data at differing time points whereas Wilcoxon Signed Rank tests were used in this instance when the data were not normally distributed. Spearman's correlation was used to assess relationships between variables. Given the number of tests performed on the same variables, Bonferroni correction was performed to adjust the P value and reduce the risk of Type I errors.

2.7.2 Primary Outcomes

The primary variables were i) the number of dogs with electrolytes concentrations within their respective reference ranges and ii) showing no clinical signs of over or under dosing of hypoadrenocorticism at each visit during the trial. Non-inferiority of the efficacy of interventional product (DOCP) vs control product (fludrocortisone) was evaluated by calculating a two-sided 95% confidence interval on the difference between the success rates in the interventional product and control product groups. The lower bound of this confidence interval had to be within the set margin of difference (22%; the non-inferiority margin), before the interventional product could be considered non-inferior to the control product. This margin of difference was defined by the lead investigator, as the percentage difference between success that would begin to cause clinical detriment to patients.

2.7.2.1 Electrolytes

The proportion of both the potassium and the sodium concentrations that were outwith their respective reference ranges at the end of each phase of the trial were compared using the Chi-square test. In addition, a Chi-square test was used to compare the sodium and potassium concentrations out with the reference range after ten days on each trial phase versus the end of each trial phase. Descriptive statistics were then used to assess whether these electrolyte abnormalities were more consistent with over or under control of hypoadrenocorticism.

The sodium and the potassium concentrations at the end of each trial phase were compared using Paired T-tests or Wilcoxon Signed Rank tests, depending on their respective distributions.

The relationship between treatment (DOCP and fludrocortisone) doses in mg/kg was compared to the sodium and the potassium concentrations using Spearman Correlation.

The number of dogs with abnormal Na/K ratios and the number of these dogs with electrolyte concentrations outwith their respective reference ranges were reviewed descriptively.

2.7.2.2 Clinical Signs

The presence of clinical signs suggestive of glucocorticoid overdose (polyuria, polydipsia, polyphagia, excessive panting and coat changes), mineralocorticoid overdose (polyuria, polydipsia, weakness) and glucocorticoid and mineralocorticoid deficiency (vomiting, diarrhoea, weakness) were reviewed at each visit with the patient's owner. If signs such as polyuria and polydipsia were considered to be more severe around day 8 to 12, with subsequent subsidence, these signs were considered more likely to be due to mineralocorticoid excess. If the signs were persistent then they were considered to be due to glucocorticoid excess and prednisolone doses were adjusted accordingly. The severities of the clinical signs were subjectively scored from 1 to 4 (1 being absent and 4 being severe). For statistical analysis, dogs were considered to have absent (score 1), mild (score 2) or clinically significant (scores 3 and 4) clinical signs. The cumulative score of each clinical sign in every patient was calculated for each phase of the trial.

Descriptive statistics were used to compare groups between the two phases of the trial and Chi-square tests were used to compare the number of dogs with clinical signs at these time points.

2.7.3 Secondary Outcomes

2.7.3.1 ACTH

The ACTH concentrations were compared at the start and at the end of each phase for each patient, using a Wilcoxon Signed Rank test. They were also compared between the end of each trial phase using this test. The ACTH concentrations were compared to the prednisolone dose in mg/kg, using Spearman Correlation. Clinical signs of glucocorticoid excess were compared to ACTH concentrations descriptively, as were signs of hypoadrenocorticism (glucocorticoid deficiency).

2.7.3.2 Renin

The renin concentrations were compared at the start and at the end of each phase, using a Wilcoxon Signed Rank test. They were also compared between the end of each trial phase using this test. The relationship between the renin concentrations and DOCP dose in mg/kg, was assessed using Spearman correlation. Clinical signs of mineralocorticoid excess were compared to renin concentrations descriptively, as were signs of hypoadrenocorticism (mineralocorticoid deficiency).

2.7.3.3 Blood pressure

The relationship between blood pressure (systolic blood pressure in mmHg) and renin concentrations, the DOCP dose (mg/kg) and the fludrocortisone dose (mg/kg) were all assessed using individual Spearman's correlations. Blood pressure was also compared between the end of each phase of the trial using a paired T-test.

For descriptive analysis, blood pressures were given a score of 0 to 2, with 0 representative of normotensive or minimal risk of target organ damage (TOD) patients (systolic pressure <160mmHg), 1 representing moderate TOD risk (systolic pressure 160-180mmHg) and 2 representing severe TOD risk (systolic pressure >180mmHg).

2.7.3.4 Haematology and biochemistry

Paired T tests and Wilcoxon Signed Rank tests were used to compare the neutrophil, lymphocyte and eosinophil counts at the end of each phase of the trial. These tests (depending on distribution) were also used to compare the urea, creatinine, albumin, glucose and calcium concentrations at the end of each phase of the trial. Although full haematology and biochemistry profiles were run, these variables were considered the most important when considering control of hypoadrenocorticism.

3 Results

3.1 Patients enrolled

3.1.1 Signalment

A total of 33 animals were recruited. The breeds, ages and genders were typical for hypoadrenocorticism when compared to those reported in the literature (Kintzer and Peterson, 1994; Melian and Peterson, 1996). Breeds included five Crossbreeds (including three Poodle crosses), five Poodles (four Standard and one Toy), four Cocker Spaniels, three Labrador Retrievers, two Border Collies, two Springer Spaniels, two West Highland White Terriers and one of each of the following: Bearded Collie, Cavalier King Charles Spaniel, German Short Haired Pointer, Great Dane, Husky, Jack Russell Terrier, Miniature Pinscher, Tibetan Mastiff, Tibetan Terrier and Utonogan. There were 16 neutered females, three entire females, 13 neutered males and one entire male. The median age was six years (range 0.5 to 12 years) and the median weight was 21.85 kg (range 5.9kg to 69kg). Tables 4 to 7 summarise the patient signalments and pre-trial data.

Table 4. Patient Signalments

Summary of patients enrolled in the trial, including those who were later excluded (*).

Case No.	Age (years)	Age Dx (years)	Breed	Sex	Weight (kg)
1	11	9	Crossbreed	FN	11.1
2	0.5	0.5	Labradoodle	F	14.8
3	10	7	Jack Russell Terrier	FN	7.6
4	4	1	Great Dane	MN	69.0
5	4	2	Cocker Spaniel	FN	12.8
6	7	3	Great Dane cross	FN	49.4
7	12	9	West Highland White Terrier	MN	10.6
8	4	2	Labradoodle	FN	40.0
9	9	6	Crossbreed	MN	27.5
10*	9	9	Bearded Collie	MN	21.2
11	7	3	Tibetan Mastiff	FN	30.8
12	5	2	WHWT	FN	6.3
13	7	3	Standard Poodle	MN	25.0
14	4	3	Standard Poodle	MN	33.1
15	7	7	Labrador	MN	33.5
16	8	7	Springer Spaniel	FN	18.9
17*	11	8	Cocker spaniel	MN	16.1
18	10	7	Working Cocker Spaniel	MN	15.6
19	9	2	Toy Poodle	MN	7.6
20	3	1	German Short Haired Pointer	MN	31.3
21	9	3	Standard Poodle	MN	33.6
22	6	4	Border Collie	F	18.5
23	5	3	Tibetan Terrier	MN	9.3
24	2	0.5	Standard Poodle	MN	24.7
25	5	4	Utanagon	F	22.90
26	3	2	Bearded collie	MN	20.30
27	1	0.5	Labrador	F	32.80
28*	7	6	Springer Spaniel	M	29.4
29	4	4	Bearded Collie cross	F	14.30
30	6	3	Cavalier King Charles Spaniel	FN	11.30
31	3	2	Husky	MN	40.20
32	9	2	Labrador	FN	42.10
33	3	2	Miniature Pinscher	FN	5.90

*Patients excluded
Dx = diagnosis
M; male, MN; male neuter, F; female, FN; female neuter

Table 5. ACTH stimulation results and fludrocortisone dose at enrolment

This table summarises the group each patient was assigned to, their initial ACTH stimulation test results, the duration of treatment prior to enrolment and the fludrocortisone dose at enrolment. Excluded patients are not shown.

Trial number	Group	Pre ACTH cortisol (nmol/L)	Post ACTH cortisol (nmol/L)	Treatment period Pre-Trial (days)	Dose of fludrocortisone at enrolment (mg, twice daily)
1	A	<7	<7	653	0.1
2	B	<10.5	<10.5	57	0.3
3	A	<14	<14	1087	0.1
4	B	14	72	1076	1.0
5	A	27	42	690	0.3
6	B	<7	<7	1243	0.6
7	A	18	14	1008	0.4
8	A	<7	<7	806	0.4
9	A	<6.9	<6.9	768	0.2
11	B	30	35	1149	0.3
12	A	<6.5	<6.5	1079	0.1
13	B	<10.5	<10.5	1270	0.3
14	B	<7	<7	602	0.4
15	B	<7	<7	89	0.3
16	A	44	45	518	0.2
18	B	19.5	22.4	1099	0.4
19	B	<5.6	<5.6	2421	0.1
20	B	27.6	27.6	840	0.4
21	B	<7	<7	2115	0.2
22	A	34	38	585	0.2
23	A	<10	<10	553	0.1
24	B	<27.6	<27.6	392	0.3
25	A	13.5	<10.5	229	0.25
26	B	<10	<10	67	0.2
27	A	7.2	7.92	379	0.25
29	A	<14	<14	125	0.1
30	A	<7	<7	1036	0.3
31	B	<6.9	<6.9	612	0.4
32	A	19	21	2516	0.1
33	A	48	22	65	0.1

Table 6. Summary of pre-trial patient data

Summary of patients, including their age at diagnosis, age at the start of the trial, signalment, fludrocortisone dose (mg/kg BID) and electrolytes at enrolment. Excluded patients not shown.

Trial No	Group A or B	Age start	Age Dx	Breed	Sex	Weight (kg)	FL dose	Na start (mmol/L)	K start (mmol/L)
1	A	11	9.6	Crossbreed	FN	11.1	0.009	154	4.1
2	B	0.5	0.5	Labradoodle	F	14.8	0.020	148	4
3	A	10	7.2	JRT	FN	7.6	0.013	152	4.5
4	B	4	1.9	Great Dane	MN	69.0	0.014	147	5.4
5	A	4	2.5	Cocker Spaniel	FN	12.8	0.023	149	4.5
6	B	7	3.8	Great Dane	FN	49.4	0.012	153	5.3
7	A	12	9.4	WHWT	MN	10.6	0.038	148	6.5
8	A'	4	2.3	Labradoodle	FN	40.0	0.022	149	4.7
9	A	9	6.9	Crossbreed	MN	27.5	0.005	155	4.8
11	B	7	3.9	Tibetan Mastiff	FN	30.8	0.010	154	4.2
12	A'	5	2.2	WHWT	FN	6.3	0.011	152	4.5
13	B	7	3.7	Standard Poodle	MN	25.0	0.012	150	4.2
14	B	4	3.2	Standard Poodle	MN	33.1	0.012	153	4.2
15	B	7	7.5	Labrador	MN	33.5	0.009	152	4.8
16	A	8	7.3	Springer Spaniel	FN	18.9	0.007	150	4.8
18	B	10	7.1	Working Cocker	MN	15.6	0.026	153	3.5
19	B'	9	2.4	Toy Poodle	MN	7.6	0.012	145	5.5
20	B	3	1.2	GSHP	MN	31.3	0.020	148	4.9
21	B	9	3.9	Standard Poodle	MN	33.6	0.013	154	4.4
22	A'	6	4.8	Border Collie	F	18.5	0.009	151	4.2
23	A'	5	3.6	Tibetan Terrier	MN	9.3	0.003	153	4.6
24	B'	2	0.1	Standard Poodle	MN	24.7	0.010	152	4.4
25	A'	5	4.6	Utanagon	F	22.90	0.017	149	4.5
26	B'	3	2.9	Bearded collie	MN	20.30	0.010	152	4.6
27	A'	1	0.8	Labrador	F	32.80	0.022	152	4.6
29	A'	4	4.5	Crossbrred	F	14.30	0.002	152	4.5
30	A'	6	3.6	CKCS	FN	11.30	0.051	145	4.3
31	B	3	2.1	Husky	MN	40.20	0.007	156	5.7
32	A	9	2.5	Labrador	FN	42.10	0.016	148	3.8
33	A	3	2.9	Miniature Pinscher	FN	5.90	0.005	146	4.7

Key:

Dx – Diagnosis

FL Dose – Fludrocortisone mg/kg twice a day

F- Female

FN – Female Neutered

M- Male

MN – Male Neutered

JRT – Jack Russel Terrier

WHWT – West Highland White Terrier

GSHP – German Short Haired Pointer

CKCS – Cavalier King Charles Spaniel

(excluded patients are not included in this table)

Table 7. Pre-trial data Group A vs B

This table shows the Pre-trial data comparison between Groups A and B. Means are shown for normally distributed data and medians for abnormal distribution. Significance is marked by an *.

		Group A	Group B	P value
Gender	Male entire	0	0	
	Female entire	4	1	
	Male neutered	3	11	
	Female neutered	8	3	
				0.013*
Age at diagnosis (years)	Mean	4.7	3.2	
	Standard deviation	2.7	2.2	
				0.099
Weight at start (kg)	Median	13.6	31.0	
	Maximum	42.1	69.0	
	Minimum	5.9	7.6	
				0.016*
Age at Day 1 (years)	Mean	6.4	5.4	
	Standard deviation	3.12	2.96	
				0.384
Fludrocortisone dose (mg/kg twice a day)	Median	0.012	0.012	
	Maximum	0.051	0.026	
	Minimum	0.002	0.007	
				0.852
Sodium at the start	Mean	150.3	151.2	
	Standard deviation	2.8	3.14	
				0.417
Potassium at the start	Median	4.5	4.5	
	Maximum	6.5	5.7	
	Minimum	3.8	3.5	
				0.900
	Total dogs	16	14	

3.1.2 Pre-existing conditions

One patient (Patient 29) had been diagnosed with pannus two days before diagnosis of hypoadrenocorticism. This patient had been treated with dexamethasone, polymixin B and neomycin sulphate eye drops (Maxitrol, Alcon) for 13 days prior to ACTH stimulation and had been started on prednisolone acetate eye drops (Pred-Forte, Allergen Ltd) two days before this test. Given the lifelong nature of this condition, the eye drops were continued throughout the trial period.

Another patient (Patient 6) had pre-existing urinary incontinence and was treated with oestradiol (Incurin, MSD Animal Health) throughout the study period. Although not on concurrent medication at the time of enrolment, one patient (Patient 15) had a pre-existing diagnosis of a recurrent malassezia otitis externa. This had resolved by the time of enrolment however it had been treated with oral ketoconazole (Sporanox, Janssen-Cilag Ltd.) and amoxicillin clavulanic-acid (Synulox, Zoetis). The patient had become unwell eight days following the introduction of ketoconazole, at which point this drug had been discontinued.

3.1.3 Concurrent medications

Several patients required treatment during the trial for conditions unrelated to hypoadrenocorticism and which weren't suspected to be associated with side effects caused by either fludrocortisone or DOCP. Three patients (1, 20 and 24) had been prescribed amoxicillin clavulanic-acid for management of pyoderma, by their primary care veterinarians. Another patient (Patient 20) was prescribed amoxicillin clavulanic-acid by its primary care veterinarian for management of a suspected urinary tract infection. Clindamycin was prescribed by the primary care veterinarian to Patient 1 for management of colitis. Malassezia otitis externa resulted in prescription of clotrimazole by the primary care veterinarian of Patient 28. Patient 19 was prescribed a short course of tramadol for management of brief hind limb lameness and Patient 25 was involved in a minor road traffic accident and had been treated with methadone. This patient was also given an increase in steroid dose (dexamethasone intravenously) following this incident for one dose. During the trial, Patient 11 was diagnosed with urinary incontinence and was started on Propalin, Vetoquinol (phenylpropanolamine).

Two patients required management for suspected trial drug related events. Patient 13 required hospitalisation, fluid therapy, maropitant, potassium supplementation and spironolactone for lethargy, anorexia, hypokalaemia and hypotension around day 10 following a DOCP injection. On one visit, buprenorphine was also prescribed for concurrent suspected pancreatitis. Patient 22 received maropitant 9 days after its third dose of DOCP as it had vomited once, was inappetent and lip smacking.

3.1.4 Patients excluded

In total, three dogs were removed from the trial. These patients had been initially enrolled in the study and therefore had been started on trial drugs. One dog was removed from the study (Patient 10) as on further evaluation of its history, it had emerged that hypoadrenocorticism had not been confirmed using an ACTH stimulation test. Instead, this condition was diagnosed purely on abnormal electrolyte concentrations (hyperkalaemia and hyponatraemia). As this patient had clinically and biochemically responded very well to steroid supplementation and as this patient was a young Bearded Collie, it was suspected that the diagnosis was correct. Therefore the patient was not weaned off glucocorticoid and mineralocorticoid therapy to confirm the diagnosis of hypoadrenocorticism as this was likely to be detrimental to its health. It was however excluded from the trial as it did not comply with the inclusion criteria.

Another (Patient 28) was excluded as during the initial trial screening, it was diagnosed with hypothyroidism with a thyroxine (T4) concentration under the detection limit of 3.2 nmol/L (reference 15-45) with a concurrent increase in thyroid stimulating hormone (TSH) of 3.54 ng/ml (0-0.61). If the low T4 was due to exogenous glucocorticoid therapy, then the TSH would not be expected to be increased (O'Neill et al., 2011, Torres et al., 1991). There was a marked increase in cholesterol (17.45mmol/L) which was considered consistent with this diagnosis. This patient was started on levothyroxine and an improvement in demeanour and a reduction in cholesterol were seen. The T4 concentrations normalised, confirming adequate management of the condition. Although no clinical signs of hypothyroidism had been described initially, the owner of this patient felt it was much brighter on thyroxine supplementation. In contrast to these results, patients on treatment for hypoadrenocorticism tend to have low T4 and TSH concentrations due to the effects that glucocorticoids have on the thyroid axis (Daminet and Ferguson, 2003, Reusch et al., 2017). The trial protocol allowed for patients with concurrent endocrinopathies, however it was a requirement that these patients be stable on treatment. This patient was excluded from the trial as although it became stable on thyroxine supplementation, it was not stable on treatment at the time of enrolment.

Finally, one dog (Patient 17) did not complete the trial as it died suddenly one week after starting. This patient was in group B and therefore was not transitioned onto DOCP. Post-mortem examination was consistent with a haemoabdomen due to a ruptured haemangiosarcoma.

3.2 Adverse events

There were two significant adverse events. The first was that one dog (Patient 17) died of a ruptured splenic haemangiosarcoma, which was thought to be unconnected to the trial. The second was Patient 13 who developed pancreatitis whilst DOCP was being administered. This dog recovered. This condition could have been linked to the administration of the DOCP treatment but this was not definitively established and the dog was able to complete the trial.

3.3 Protocol deviations

There were two significant protocol deviations. One was that during the study period the branded preparation of fludrocortisone acetate (Florinef®, Bristol) became unavailable. Therefore a generic fludrocortisone tablet formulation (Aspen Pharmaceuticals) was used instead.

Initially the study protocol had stated that non-inferiority would be determined by treatment success at each visit during each phase of the trial (section 2.1) and that success was patients showing no signs of over or under dosing of each drug. However during the study, the success criteria was changed. Success was defined as patients showing no signs (clinical or electrolyte abnormalities) suggestive of steroid under dose and at the end of the trial only.

3.4 Study results

3.4.1 Study Design

There was one significant Protocol Amendment during this trial which was required following withdrawal of Florinef® (Bristol Squibb Myers) from the market. Generic fludrocortisone (Aspen) was administered instead which was significantly more expensive. Therefore dogs in Group B who were treated with fludrocortisone did not require a washout period before starting the second phase of the trial and therefore were treated with three months of fludrocortisone followed immediately by three months of DOCP with prednisolone. Figure 1b illustrates this change in study design.

During the trial it became apparent that the starting dose of DOCP (mg/kg) was too high for a significant number of dogs, meaning several dose reductions were required. Therefore unscheduled visits were arranged ten days after any dose change (see section 3.3.4).

Given this apparent over treatment with DOCP in many patients, the criteria for non-inferiority was adjusted slightly. The initial Study Protocol had stated that treatment success would be classed as: “the percentage of dogs reported as having normal concentrations of sodium and potassium or having no clinical signs at each observation point on the trial” (see section 2.1). However, many dogs had clinical signs and electrolyte concentrations suggestive of over treatment. This was not considered to reflect on treatment success, as these patients showed no signs of under treatment or treatment failure. Therefore the classification of treatment success was instead considered to be: “the percentage of dogs reported as having no hyperkalaemia or hyponatraemiam with no clinical signs of hypoadrenocorticism at the end of each phase of the trial” (see section 3.3.8). The end of the trial was used, instead of each observation point, to allow dose adjustments of both treatments to be made before considering success.

3.4.2 Groups

There were 16 dogs in Group A (DOCP followed by fludrocortisone) and 14 dogs in Group B (fludrocortisone followed by DOCP). When the patient data from the two groups (A and B) were compared it was found that there was a significant difference in weight between them (Mann-Whitney U test, P= 0.016). In addition, there was a significant difference between the gender distribution (Chi-square Test, P= 0.013) with more male neutered dogs in Group B and more females in Group A. There were no significant differences between the two groups in the dose of fludrocortisone per kg of weight (Mann-Whitney U test), the age at the start of the trial, the age at diagnosis, or the time between diagnosis and the start of trial (all Independent T Test). There was no significant difference in sodium and potassium concentrations between the two groups at the beginning of the trial (using an Independent T Test and Mann-Whitney U Test respectively). Drug doses were given in mg/kg and the difference in gender distribution was not considered to affect the trial results. Therefore, the two groups (A and B) were combined to give one group and the two phases of the trial, the DOCP phase and the fludrocortisone phase, were considered separately.

3.4.3 Visits

The predicted total number of visits according to the original protocol was 300. The total number of visits achieved was 298. Two dogs did not attend one appointment each. When the A visits were excluded, the total number of visits should have totalled 240. The total number of these visits that were within 3 days of the expected time (i.e. 8-10 or 28, 56, 84 days) for the B, C, D and E visit was 214 (89%). Table 8 and Figure 2 summarise the days on which each visit took place.

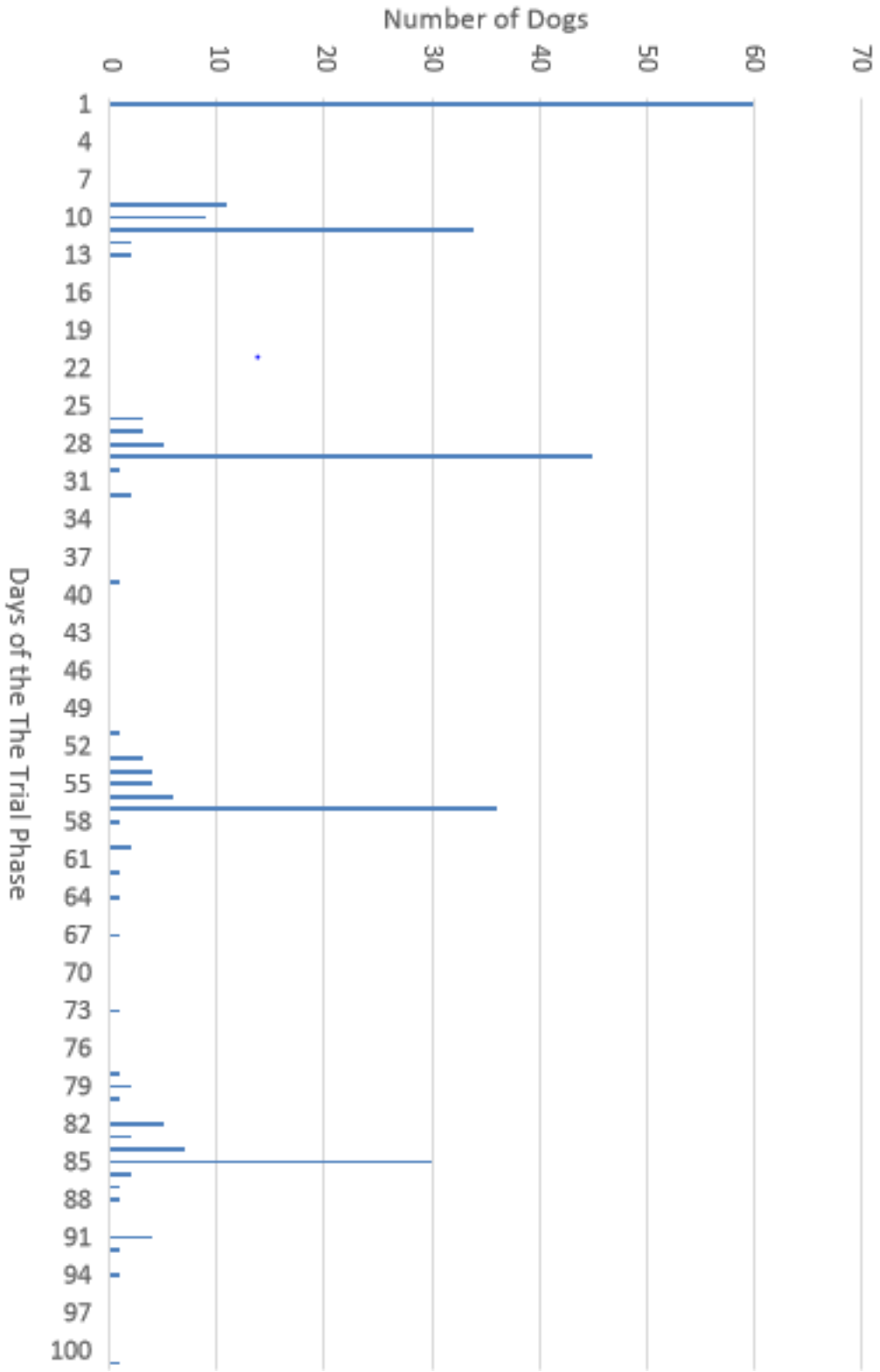
Table 8. Summary of visits on each day of the trial

The number of visits that occurred on each day after starting each phase of the trial for all 30 dogs. Original target dates are highlighted in blue.

Days since start of phase	Number of visits	Days since start of phase	Number of visits	Days since start of phase	Number of visits	Days since start of phase	Number of visits
0 (A visit)	60						
1	0	26	3	51	0	76	0
2	0	27	5	52	3	77	1
3	0	28(C visit)	45	53	4	78	2
4	0	29	1	54	4	79	1
5	0	30	0	55	6	80	0
6	0	31	2	56(D visit)	36	81	5
7	0	32	0	57	1	82	2
8	11	33	0	58	0	83	7
9	9	34	0	59	2	84 (E visit)	30
10 (B visit)	34	35	0	60	0	85	2
11	2	36	0	61	1	86	1
12	2	37	0	62	0	87	1
13	0	38	1	63	1	88	0
14	0	39	0	64	0	89	0
15	0	40	0	65	0	90	4
16	0	41	0	66	1	91	1
17	0	42	0	67	0	92	0
18	0	43	0	68	0	93	1
19	0	44	0	69	0	94	0
20	0	45	0	70	0	95	0
21	0	46	0	71	0	96	0
22	0	47	0	72	1	97	0
23	0	48	0	73	0	98	0
24	0	49	0	74	0	99	0
25	3	50	1	75	0	100	1

Figure 2. Graphical representation of visits which took place on each day of each phase of the trial

Bar chart of the number of dogs presenting for a visit on each day of each phase of the trial. As there were two phases of the trial, this meant the maximum number of dogs was 60, rather than 30. Day 1 was the start of each phase (and does not include the washout phase). As each phase of the trial was 12 weeks long, the scheduled E visit would have been anticipated on day 84. The blue bar represents the number of dogs.



3.4.4 Unscheduled Visits

There were 38 additional visits made outwith the protocol. Thirty-five of these were during the DOCP phase and three were during the fludrocortisone phase. Ten of these were conducted ten days following a DOCP dose change, to check the electrolytes were within the normal range and to assist in assessment of the dose change. Nine visits were due to the DOCP injection being brought forward or delayed given reduced or prolonged duration of DOCP respectively (see section 3.3.5). One dog (Patient 13) required seven unscheduled visits whilst on the DOCP phase to manage signs of mineralocorticoid overdose (hypokalaemia, hypernatraemia, hypertension) and pancreatitis (diagnosed based on an abnormal SnapPL^{TM,} IDEXX test and a hypoechoic pancreas on ultrasound). The remaining visits were primarily to assess the patients when they seemed unwell in any way, to ascertain if the illness was related to hypoadrenocorticism and the associated treatment.

3.4.5 Doses of Treatment Drugs

During the trial, 25 dogs received no change in their fludrocortisone dose, three dogs had a minor dose reduction and two dogs received a slight increase in their dose. One of the dogs that required a dose increase had been on concurrent prednisolone therapy and this treatment was stopped at the time of the dose increase. The three dogs who had fludrocortisone dose decreases were showing signs of steroid overdose such as polyuria and polydipsia. The median dose of fludrocortisone at the end of the fludrocortisone phase of the trial was 0.012mg/kg (range 0.002 to 0.040) twice daily. This was in comparison to a median dose of 0.015mg/kg (range 0.002 to 0.050) twice daily at the start of the fludrocortisone phase.

Twenty-eight dogs required a dose reduction at least once during the DOCP phase of the trial and two dogs required a dose increase. Fifteen dogs (on 25 occasions) received injections which were not given 28 days following the previous injection. On ten of these occasions, this was due to owner compliance and appointment scheduling. These injections were given one day early or one day late other than on three occasions when it was given up to four days early or late. Two dogs (Patients 7 and 21) required DOCP injections every 25 days as the duration of action of DOCP was not considered to be long enough in these patients, resulting in hyperkalaemia. Four dogs required their injections to be given three to seven days late given on going hypokalaemia or hypernatraemia at 28 days. Despite consecutive dose reductions, the duration of action of DOCP in these patients seemed to be prolonged. The median dose of DOCP at the end of the DOCP phase of the trial was 1.74mg/kg (range 1.09 to 2.6). All patients were started on 2.2mg/kg of DOCP subcutaneously.

Dose reductions in prednisolone were required in 22 dogs, the dose was unchanged in eight dogs and increased in two dogs. Dose reductions were required due to the presence of a combination of polyuria, polydipsia, panting, polyphagia and coat changes. Prednisolone dose increases were made in two patients who had electrolytes within the normal reference range but seemed weak. No patients were on prednisolone at the end of the fludrocortisone phase of the trial. The median dose of prednisolone at the end of the DOCP phase of the trial was 0.13mg/kg once a day (range 0.02-0.35). This was in comparison to a dose of 0.21mg/kg (range 0.17 to 0.35) at the start of the trial.

3.4.6 Primary Outcome Measures

3.4.6.1 Electrolytes

Electrolytes were assessed at each visit, regardless of the phase of trial. The sodium was not above reference at any point on the trial but was below reference on 5 occasions (range 111.8 – 135mmol/L; normal reference range 136-159). These were all during the fludrocortisone phase of the trial; twice during the D visit and three times during the E visit. The result of 111.8mmol/L was considered to be spurious because the patient was showing no signs of disease and a hyponatraemia of this magnitude would be considered to be a clinical emergency. Therefore it was excluded from all further analysis (revised range 134.3 – 135mmol/L; normal reference range 136-159).

In contrast, the potassium concentrations were low predominantly during the DOCP phase of the trial. The potassium was low on 13 occasions during the trial (range 2.9 – 3.3mmol/L; normal reference range 3.4 – 5.8): 12 of these times during the DOCP phase and once during the fludrocortisone phase. The low potassium concentration during the fludrocortisone phase was at visit A1, following an injection of DOCP 56 days earlier. During the DOCP phase, the potassium was low at the A visit once, the B visit three times and four times at both the D and E visits. The potassium concentration was increased on one occasion (5.9 mmol/L) at the A visit of the DOCP phase, whilst the patient was on treatment with fludrocortisone. Table 9 and Figures 3, 4 and 5 show the range of sodium and potassium results during the trial.

Table 9. Minimum, maximum and median sodium potassium concentrations during the trial. Mean and standard deviation of sodium:potassium ratios.

The range of sodium and potassium results found on each visit, during each phase of the trial. Range and medians were used for data which was not normally distributed and mean and standard deviation for data which was normally distributed.

		Sodium (mmol/l)			Potassium (mmol/l)			Sodium:potassium Ratio	
Visits	**Count**	**Min**	**Max**	**Median**	**Min**	**Max**	**Median**	**Mean**	**Standard Deviation**
Fludro-cortisone phase									
A	30	139.9	153.5	147.4	3.1	5.3	4.2	35.3	4.99
B	28	142.5	152.2	147.2	3.5	5.7	4.4	33.3	4.36
C	30	136.1	152.4	146.8	3.5	5.3	4.5	33.8	4.19
D	29	134.8	152.3	145.1	3.7	5.3	4.4	32.9	3.56
E	30	111.8	150.2	146.9	3.4	5.6	4.6	32.7	4.25
DOCP phase									
A	30	138.8	151.7	146.7	3.5	5.9	4.4	32.9	4.07
B	30	143.6	154.4	149.0	2.9	4.9	3.8	39.2	4.81
C	30	138.0	152.9	148.6	3.3	5.4	3.8	38.3	4.32
D	30	143.4	153.6	148.9	3.1	5.2	3.8	39.0	4.88
E	30	145.0	153.1	148.3	3.0	4.4	3.7	40.0	3.70

Figure 3. Proportion of electrolytes out with the normal reference interval at ten days into and at the end of each trial phase

Electrolyte results (sodium and potassium) at difference phases of the trial. More specifically, it shows the proportion of results above the reference range, below the reference range and within the reference range. The sodium reference range was 136-159mmol/L and the potassium reference range was 3.4-5.8mmol/L. Some bars equate to less than 30 due to results not being obtained for a small number of patients.

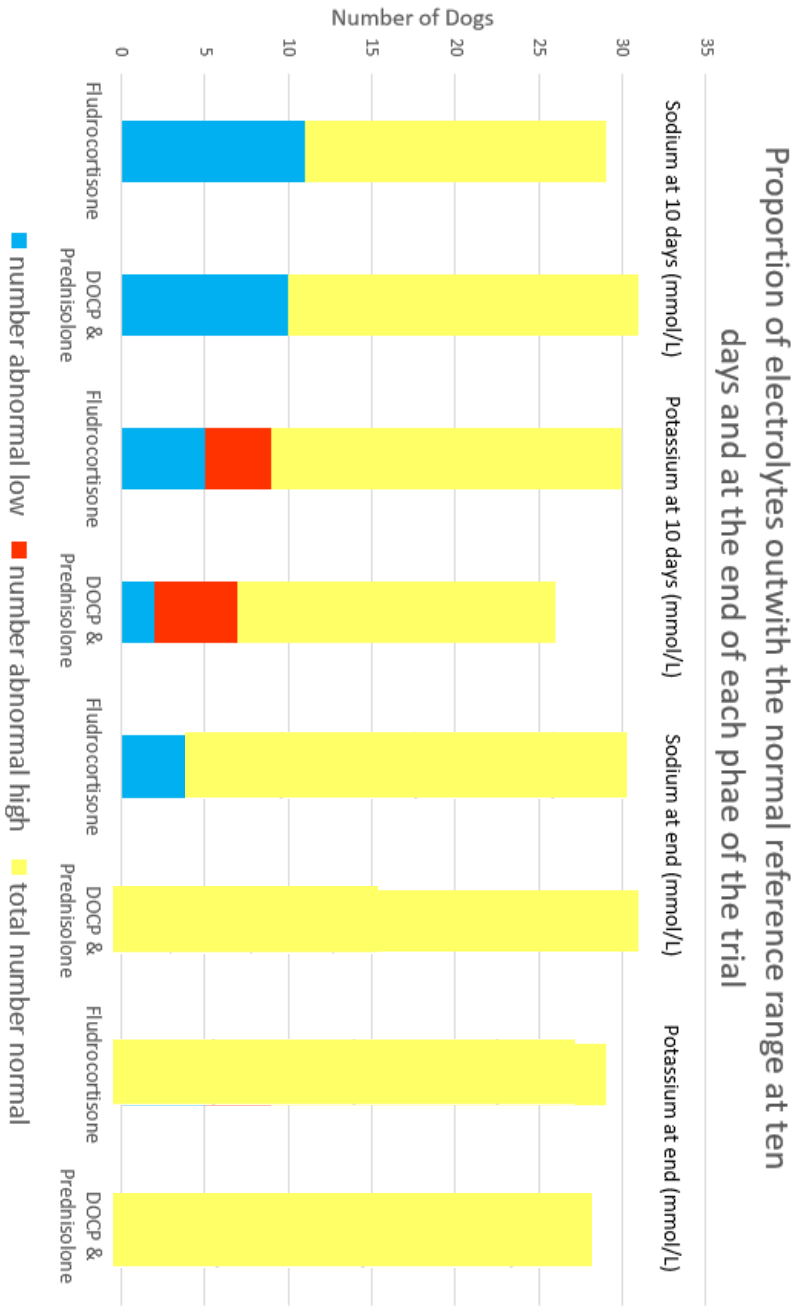


Figure 4. Sodium concentrations at each visit during trial, on each treatment

Box and whisker plot of the sodium concentrations at each visit during each phase of the trial. The coloured box represents the interquartile range (Q3 to Q1; 50% of the data set) with the solid bar within the box representing the median result (Q2). The whiskers show the remaining data (the 5th to the 95th percentiles). Outliers are marked by a symbol. Outliers are observations which are at least 1.5 times the interquartile range (Q3-Q1) from the edge of the box.

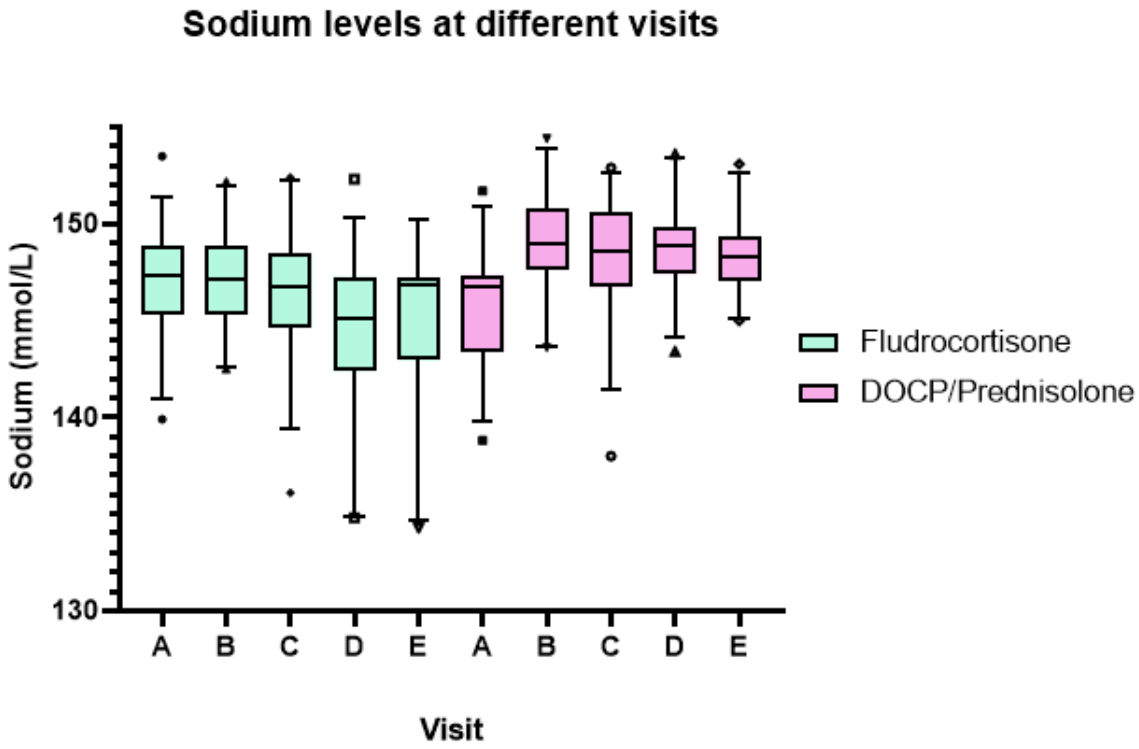
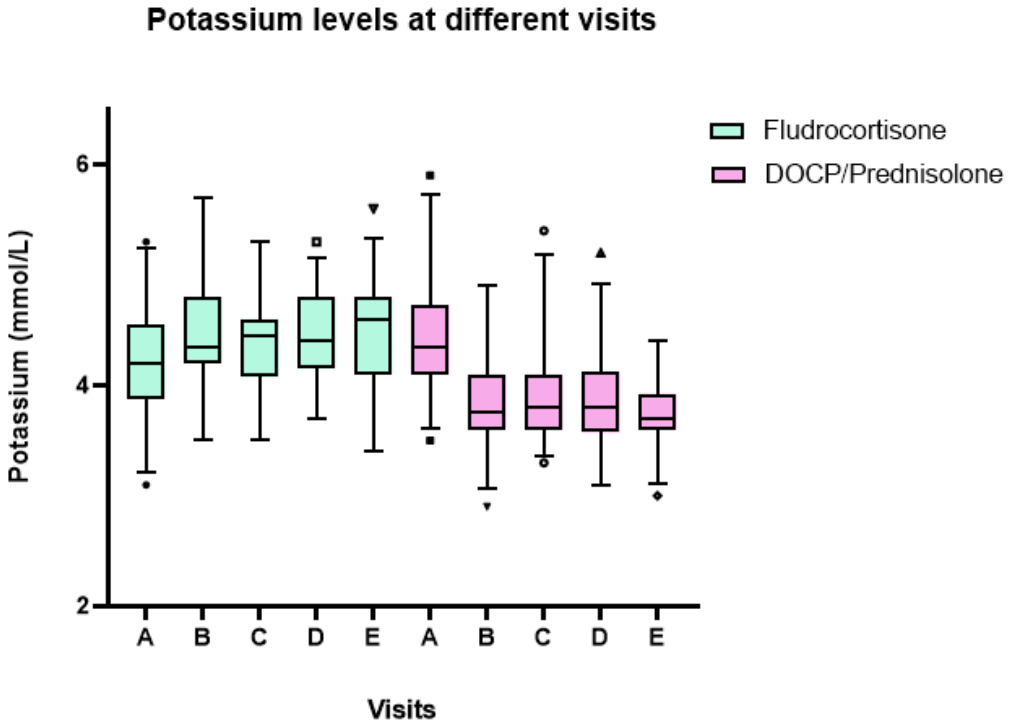


Figure 5. Potassium concentrations at each visit during trial, on each treatment

Box and whisker plot of the potassium concentrations at each visit, during each phase of the trial. The coloured box represents the interquartile range (Q3 to Q1; 50% of the data set) with the solid bar within the box representing the median result (Q2). The whiskers show the remaining data (the 5th to the 95th percentiles). Outliers are marked by a symbol. Outliers are observations which are at least 1.5 times the interquartile range (Q3-Q1) from the edge of the box.



Comparing the two phases of the trial using a Chi-Square test, there was a significant difference (P= 0.020) between the proportions of potassium concentrations which were below the reference range at the end of the DOCP phase (17%), compared to the end of the fludrocortisone phase (0%). Using this test, there was no significant difference between proportions of the sodium or potassium concentrations out with the reference range after one vs. three months of either treatment (DOCP phase vs fludrocortisone phase). Table 10 and Figure 3 show the proportion of electrolytes outwith the normal reference range.

Table 10. Proportion of electrolytes out with the normal reference interval at the B and E visits

A summary of the number of electrolytes out with the normal reference range ten days into each phase of the trial and at the end of each phase of the trial

	Na B FL	Na B DOCP	K B FL	K B DOCP	Na E FL	Na E DOCP	K E FL	K E DOCP
Total number	25	25	25	25	24	25	24	25
Number abnormal low	7	4	3	5	4	4	3	4
Number abnormal high	0	0	1	1	0	0	1	1
Total number normal	18	21	21	19	20	21	20	20
Total number abnormal	7	4	4	6	4	4	4	5
P value		NS		NS		NS		**0.02***

*Significant

NS. not significant

Key

FL – Fludrocortisone phase

DOCP – DOCP and prednisolone phase

B – B visit; ten days into the phase

E – E visit; at the end of the phase

K – Potassium mmol/L

Na – Sodium mmol/L

There was a significant difference between the sodium concentrations at the end of the fludrocortisone phase (mean 144mmol/L; SD 7.2) vs the end of the DOCP phase (mean 148.2mmol/L; SD 1.8) (Paired T test; P=0.002) and between the potassium concentrations (Wilcoxon Signed Rank test; P=0.000) at these points (potassium at end of fludrocortisone phase mean 4.47mmol/L; SD 0.53 and at end of DOCP phase mean 3.73mmol/L; SD 0.33). The sodium concentrations after one month (mean 146mmol/L; SD 3.3) vs after three months of fludrocortisone (144mmol/L; SD 7.2) were significantly different (Paired T test; P=0.017) however there was no significant difference between the sodium concentrations after one month vs after three months of DOCP (DOCP phase) or in the potassium concentrations at these time points on the fludrocortisone or DOCP phases of the trial.

There was significant correlation between the DOCP dose (mg/kg) and the potassium concentrations (Spearman Correlation; r=0.000) at the end of the DOCP phase (Figure 6). There was no correlation (using Spearman Correlation) between the DOCP dose (mg/kg) and the sodium concentrations or the fludrocortisone dose (mg/kg) with either the sodium or the potassium concentrations at the end of each phase of the trial (Figure 7). Only one patient with hypokalaemia showed clinical signs of lethargy and weakness.

Figure 6. Relationship between DOCP dose and sodium and potassium concentrations

Scatterplot of the relationship between the DOCP dose (x axis) and the sodium (left y axis) and potassium (right y axis) results. Using Spearman Correlation, only the potassium results were significantly (positively) correlated and the r value is shown.

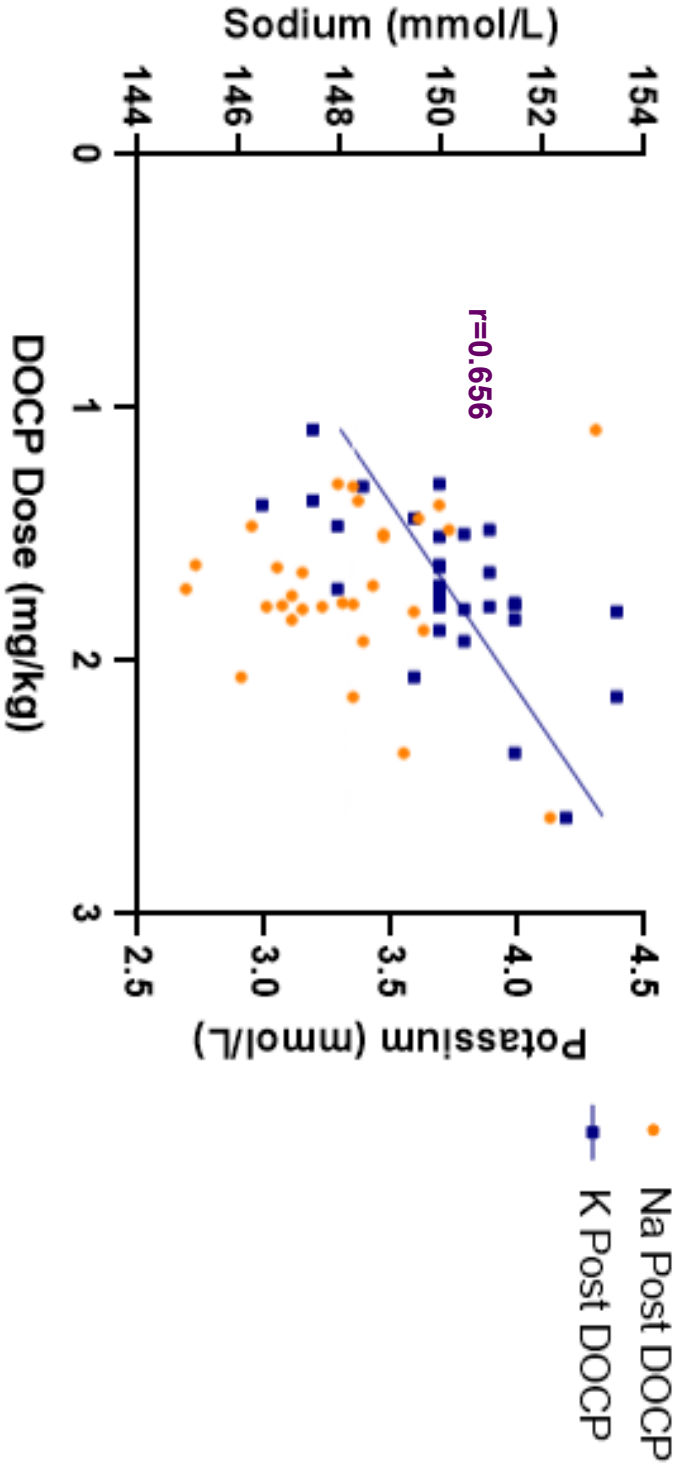
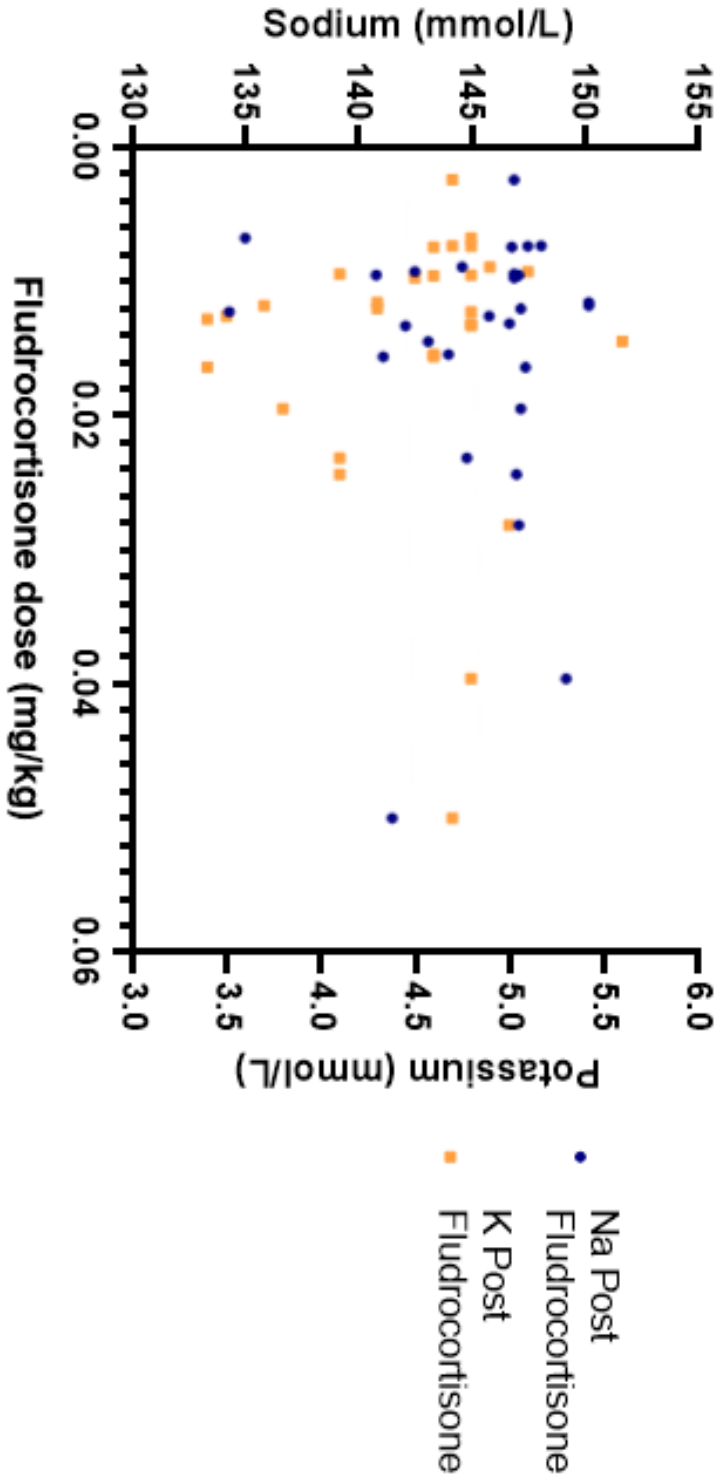


Figure 7. Relationship between fludrocortisone dose and sodium and potassium concentrations

Scatterplot of the relationship between the fludrocortisone dose (x axis) and the sodium (left y axis) and potassium (right y axis) results. Using Spearman Correlation, these results were not significant.

*The sodium result of 111.8mmol/L for omitted from analysis



The sodium:potassium ratio was assessed as a potential indicator of mineralocorticoid control and overall, was not found to be useful. During the scheduled visits, when a normal range of 27 – 32 was used, there was an increase in the sodium:potassium ratio on 207 occasions. Only 60 of these visits (29%) were during the fludrocortisone phase, not including the first visit of the phase when DOCP had been given 8 weeks earlier. This reduced to 12 occasions when an upper limit of 42 was used and 3 when an upper limit of 46 was used. The ratio was low on seven visits when the electrolytes were within the normal range. This would have incorrectly led to a dose adjustment if using sodium:potassium ratios as a marker of control (see Table 3). In addition, on six occasions the sodium:potassium ratio was normal whilst the sodium was low. This could have resulted in continuation of a mineralocorticoid dose which was not high enough (during the fludrocortisone phase). There was agreement with the sodium:potassium ratio with the respective individual sodium and potassium concentrations 21 times when the potassium was low, three times when the sodium was low and once when the potassium was high.

3.4.6.2 Clinical Signs

3.4.6.2.1 Clinical Signs of Glucocorticoid Excess

Polyuria, polydipsia, polyphagia, excessive panting and coat changes were used as clinical markers of glucocorticoid excess. Using a Chi-Square test, there was no significant difference between the proportion of dogs with and without clinical signs at the end of each phase of the trial or after one and three months of either treatment (DOCP and prednisolone or fludrocortisone).

At the start of the fludrocortisone phase, the cumulative subjective score (see section 2.7.2.2) was 93 and at the end of this phase it was 77. For the DOCP phase, the cumulative subjective score was 97 and at the end of the DOCP phase, the cumulative subjective score was 144, suggesting an increase in clinical signs of glucocorticoid overdose with DOCP and prednisolone treatment. Figure 8a summarises this data.

Figure 8. Clinical signs of treatment overdose

Figure 8a. Clinical Signs of glucocorticoid overdose

Bar chart of the clinical signs seen on each phase of the trial, as the A, B and E visits. The severities of the clinical signs were subjectively scored from 1 to 4 (1 being absent and 4 being severe). As there were 30 patients, this meant the minimum score for each clinical signs was 30 and the maximum score for each clinical sign was 120. Polyuria and polydipsia are included although these signs could also be due to mineralocorticoid overdose.

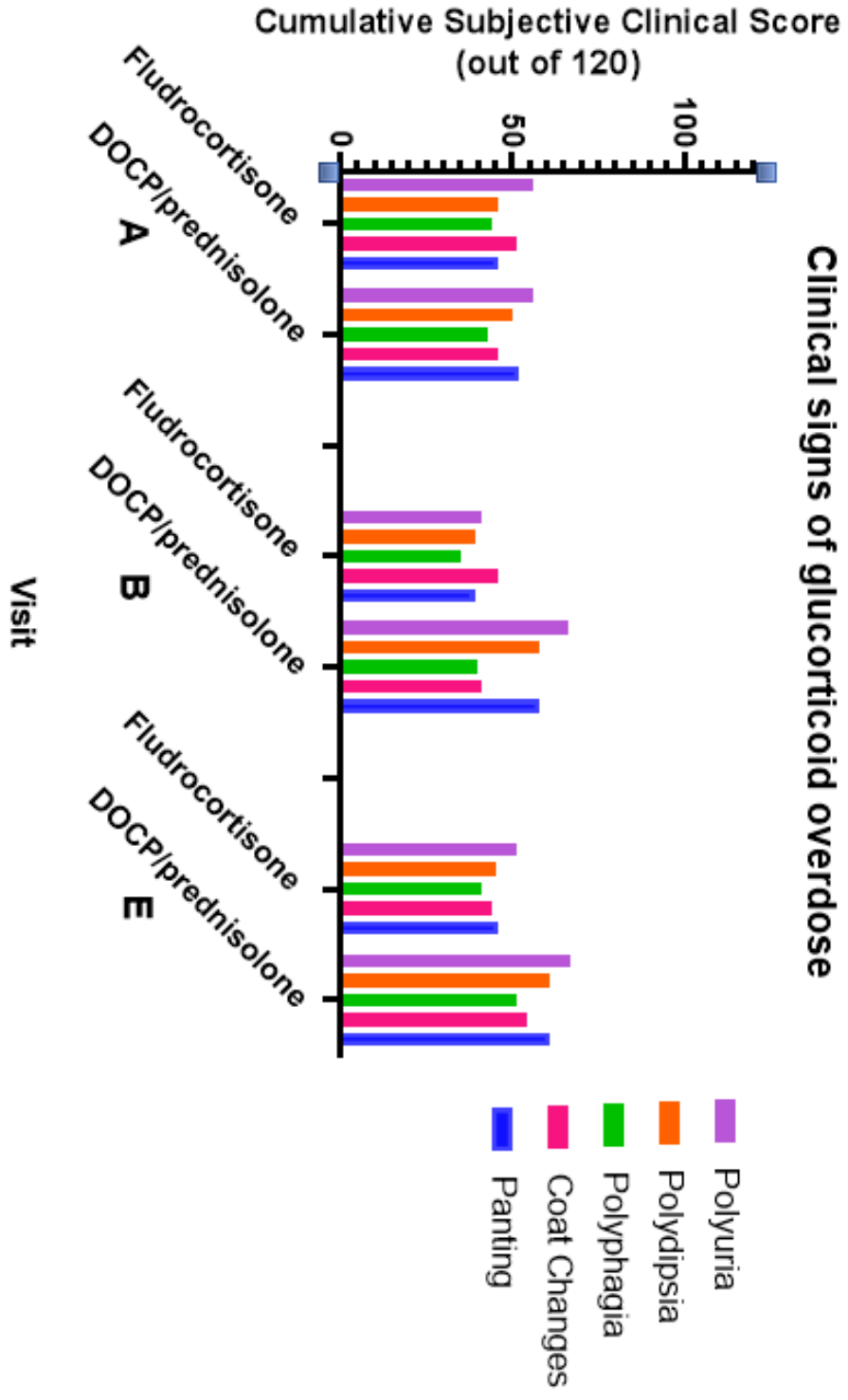
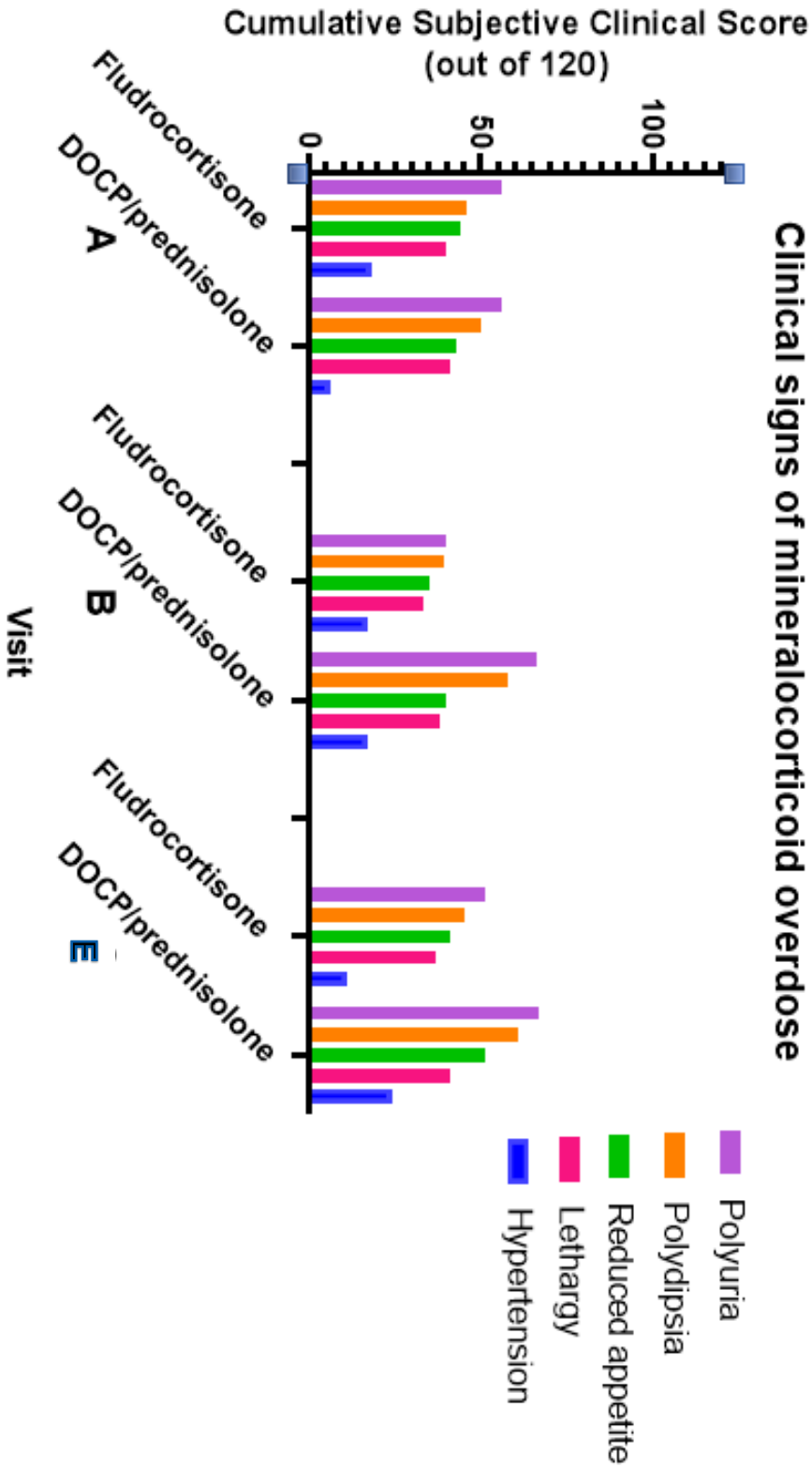


Figure 8b. Clinical Signs of mineralocorticoid overdose

Bar chart of the clinical signs seen on each phase of the trial, as the A, B and E visits. The severities of the clinical signs were subjectively scored from 1 to 4 (1 being absent and 4 being severe). As there were 30 patients, this meant that the minimum score for each clinical signs was 30 and the maximum score for each clinical sign was 120. Polyuria and polydipsia are included although these signs could also be due to glucocorticoid overdose.



3.4.6.2.2 Clinical Signs of Mineralocorticoid Excess

Polyuria, polydipsia, weakness and hypertension were used as clinical markers of mineralocorticoid excess. Polyuria and polydipsia (PU/PD) were considered separately from the other signs given that they are hard to distinguish from signs of glucocorticoid excess. Using a Chi-Square test, there was a significant difference (P=0.014) in the proportion of dogs with PU/PD ten days after the first DOCP injection (B visit DOCP phase, 79%) compared to 10 days after starting the fludrocortisone phase (B visit fludrocortisone phase 46%). There was however no difference in the proportion of dogs with PU/PD at the B visit and E visit of either phase of the trial.

When considering hypertension and weakness, there was a significant difference in the proportion of dogs with the presence of clinical signs (Chi-Square; P=0.035) at the end of the fludrocortisone phase (47%) compared to the end of the DOCP phase (73%). There was no significant difference in the proportion of dogs with clinical signs at the B and E visit on either phase of the trial.

Polyuria and polydipsia could be considered signs of overdose of either treatment. Considering this sign only, the cumulative score (see section 2.7.2.2) was 102/240 at the start of the FL phase and 96/240 at the end, compared to 106/240 and 128/240 respectively for the DOCP phase. For the remaining clinical signs of glucocorticoid overdose (polyphagia, coat changes and panting), the cumulative subjective score was 141/360 at the start of the FL phase and 131/360 at the end, compared to 141/360 and 166/360 respectively for the DOCP phase (Figure 4a). For remaining clinical signs of mineralocorticoid overdose (reduced appetite, lethargy and hypertension), the cumulative subjective score was 102/360 at the start of the FL phase and 89/360 at the end compared to 90/360 and 116/360 respectively for the DOCP phase (Figure 4b). These results suggest an increase in clinical signs of glucocorticoid and mineralocorticoid overdose with DOCP and prednisolone treatment compared to FL treatment.

3.4.6.2.3 Clinical signs of hypoadrenocorticism

One patient (Patient 13) presented with signs which could have been considered to be associated with untreated hypoadrenocorticism (vomiting, lethargy and weakness). This occurred 6 to 16 days after each injection of DOCP and was also associated with hypokalaemia (nadir of 3.0 mmol/L; reference range 3.4-5.8) and hypertension (maximum mean systolic pressure 186 mmHg, enrolment visit systolic pressure 134 mmHg). Therefore, it was suspected that these signs were associated with mineralocorticoid overdose alone, rather than mineralocorticoid or glucocorticoid overdose. These signs resolved post injection following DOCP dose reductions.

3.4.7 Secondary Outcome Measures

3.4.7.1 ACTH concentrations

ACTH was measured at the start and end of the DOCP phase in all dogs and at the start and end of the fludrocortisone phase in all but one dog. The ACTH concentration increased in two dogs, stayed the same in five and decreased in 23 dogs during the DOCP phase. During the fludrocortisone phase, it increased in 13 dogs, stayed the same in eight dogs and decreased in eight dogs. The ACTH concentration was undetectable in two dogs at the start of the DOCP phase (one group A and one group B) and 15 dogs at the end of the DOCP stage (six group A and nine group B). It was undetectable in only four dogs at the start of the fludrocortisone phase (all group B) and three dogs at the end of the fludrocortisone phase (two group A and one group B).

The ACTH concentrations were significantly lower at the end of the DOCP phase (Wilcoxon Signed Rank test; P=0.000) when compared to the start but not between the start and end of the fludrocortisone phase. They were also significantly lower (Wilcoxon Signed Rank test; P=0.000) at the end of the DOCP phase compared to the end of the fludrocortisone phase. There was no correlation between the ACTH concentrations and the prednisolone dose (mg/kg) on the DOCP phase (Spearman Correlation). Although statistical analysis was not performed, there appeared to be no obvious relationship between the severity of clinical signs and complete suppression of ACTH. The ACTH concentrations are summarised in Table 11 and Figure 9.

Table 11. Summary of ACTH and renin results at the end of each phase of the trial

This table shows the ACTH and renin results at the start and end of each phase of the trial. Each individual patient and which group they were assigned to is shown.

		ACTH (pg/ml)				Renin (ng/ml/h)			
Patient	**Group**	**Pre DOCP**	**Post DOCP**	**Pre FL**	**Post FL**	**Pre DOCP**	**Post DOCP**	**Pre FL**	**Post FL**
1	A	14.4	15.3	523	560	0.44	0.15	0	0
2	B	206	2.5	384	126	2.29	0.44	20.5	24.79
3	A	309	61	281	463	1.78	0	0.07	0.07
4	B	175	39	105	175	15.98	1.63	15.84	15.98
5	A	128	2.5	24	41	0.44	0.15	0	0
6	B	28	5.7	2.5	28	2.29	0.07	4.96	2.29
7	A	129	2.5	*	21	7.47	0.52	*	2.6
8	A	178	20	112	286	2.74	0	0.3	8.29
9	A	76	2.5	162	95	0.89	0	0	0.15
11	B	310	507	218	310	0	0	0.15	0
12	A	2.5	2.5	312	2.5	2.89	0.07	1.18	17.69
13	B	5.1	2.5	2.5	5.1	0.74	0.07	0.07	0.74
14	B	226	2.5	256	226	2	0	10.58	2
15	B	81	8.6	12	81	2.4	0	0	2.4
16	A	19.1	5.1	142	193	14.43	0.07	0.07	0
18	B	2.5	2.5	2.5	2.5	0	0.07	0.15	0
19	B	196	6.9	363	196	1.92	0.07	2.89	1.92
20	B	13.1	2.5	12.7	13.1	11.91	0.07	6.51	11.91
21	B	580	2.5	469	580	0	0	0.3	0
22	A	217	21	281	186	12.65	0.52	0.59	25.46
23	A	256	8.6	436	196	18.13	0.07	0.3	9.69
24	B	63	2.5	386	63	7.55	0.07	14.95	7.55
25	A	108	31	28	102	8.14	0.15	0	1.48
26	B	384	2.5	109	384	14.8	1.48	17.83	14.8
27	A	307	89	317	315	5.7	0.15	0.15	6.51
29	A	9.2	2.5	79	2.5	23.46	0	0	46.9
30	A	230	73	298	266	2.96	0.30	1.7	2.22
31	B	33	2.5	2.5	33	14.5	0	1.41	14.5
32	A	243	2.5	52	241	0.59	0	0.15	0.22
33	B	2.5	212	9.7	221	19.83	5.70	9.69	19.61
	Median	128.5	3.8	142	180.5	2.815	0.07	0.3	2.345
	Minimum	2.5	2.5	2.5	2.5	0	0	0	0
	Maximum	580	507	523	580	23.46	19.61	20.5	46.9
	P value		0.000		0.641		0.000		0.137

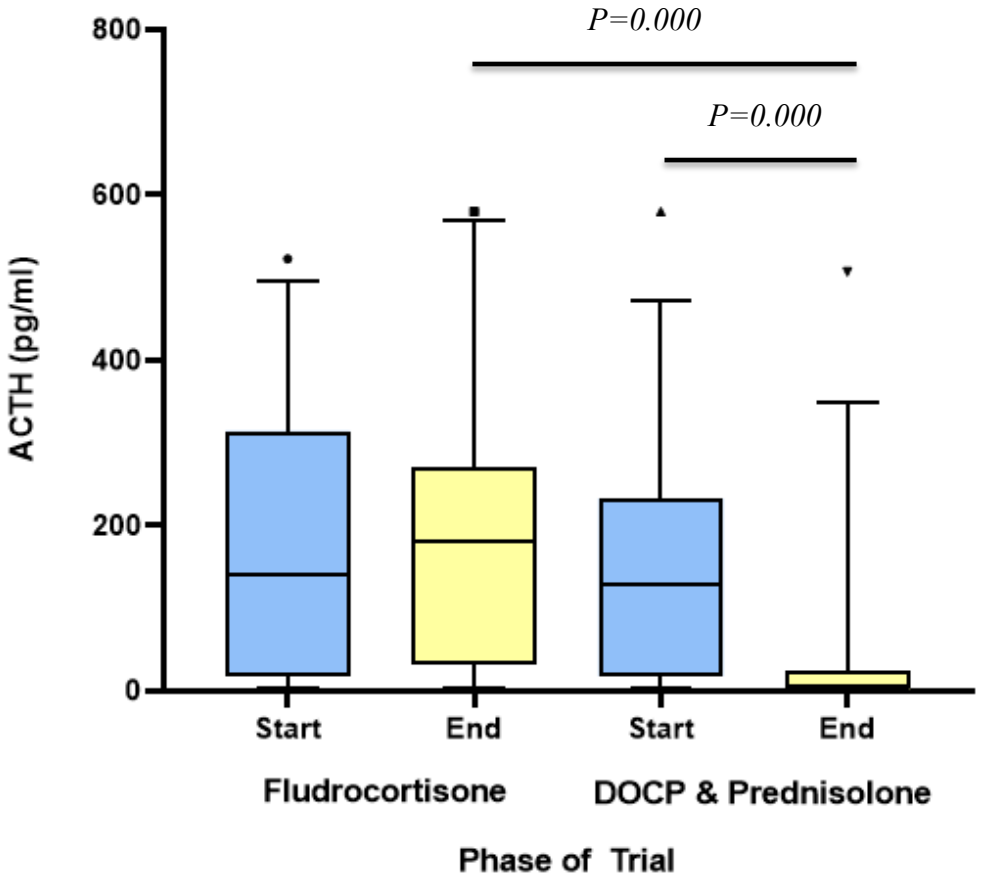
Key

FL – Fludrocortisone phase; DOCP – DOCP and prednisolone phase

** - data not collected*

Figure 9. Summary of ACTH results at the start and end of each trial phase

Box and whisker plot of the ACTH concentrations at the start and end of each phase of the trial. The coloured box represents the interquartile range (Q3 to Q1; 50% of the data set) with the solid bar within the box representing the median result (Q2). The whiskers show the remaining data (the 5th to the 95th percentiles). Outliers are marked by a symbol. Outliers are observations which are at least 1.5 times the interquartile range (Q3-Q1) from the edge of the box.

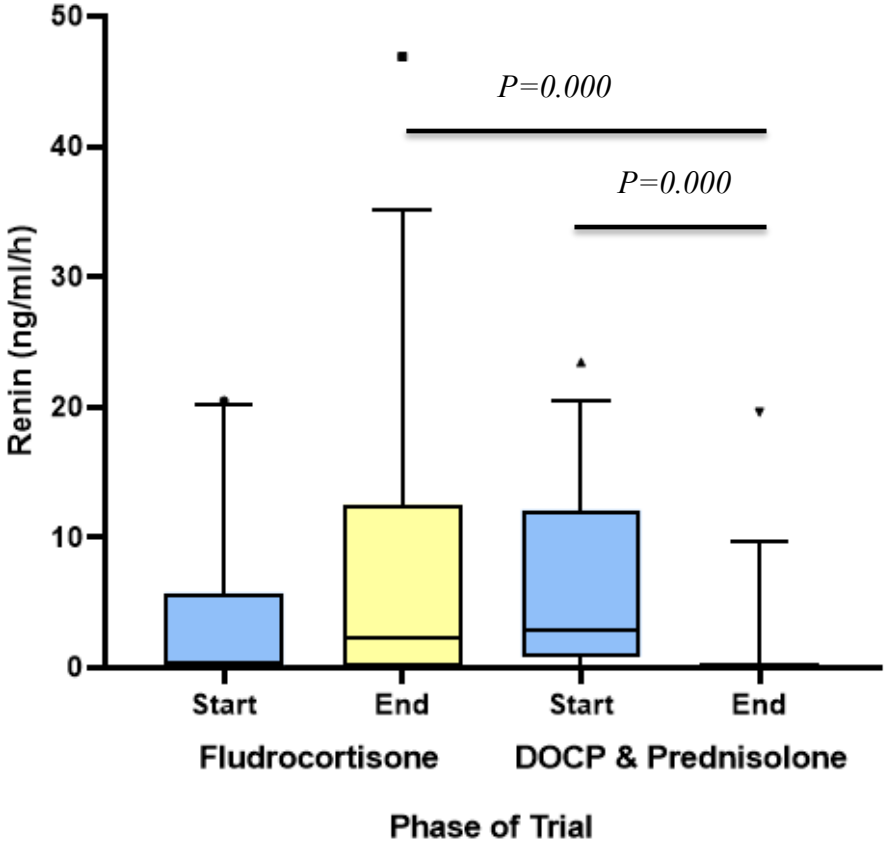


3.4.7.2 Plasma renin activity

Renin was measured at the start and end of the DOCP phase in all dogs and at the start and end of the fludrocortisone phase in all but one dog. During the DOCP phase, the renin concentrations stayed the same (n=3) or decreased (n=27). During the fludrocortisone phase, it increased in 14 dogs, stayed the same in six dogs and decreased in nine dogs. The renin concentrations were undetectable in three dogs at the start of the DOCP phase (all group B) and in 19 dogs by the end of the DOCP phase (eight group A and 11 group B). Nine dogs had an undetectable renin at the start of the fludrocortisone phase (seven group A and two group B) and six had an undetectable concentration at the end of the fludrocortisone phase (four group A and two group B). The renin results are summarised in Table 11 and Figure 10.

Figure 10. Summary of renin results at the start and end of each phase of the trial

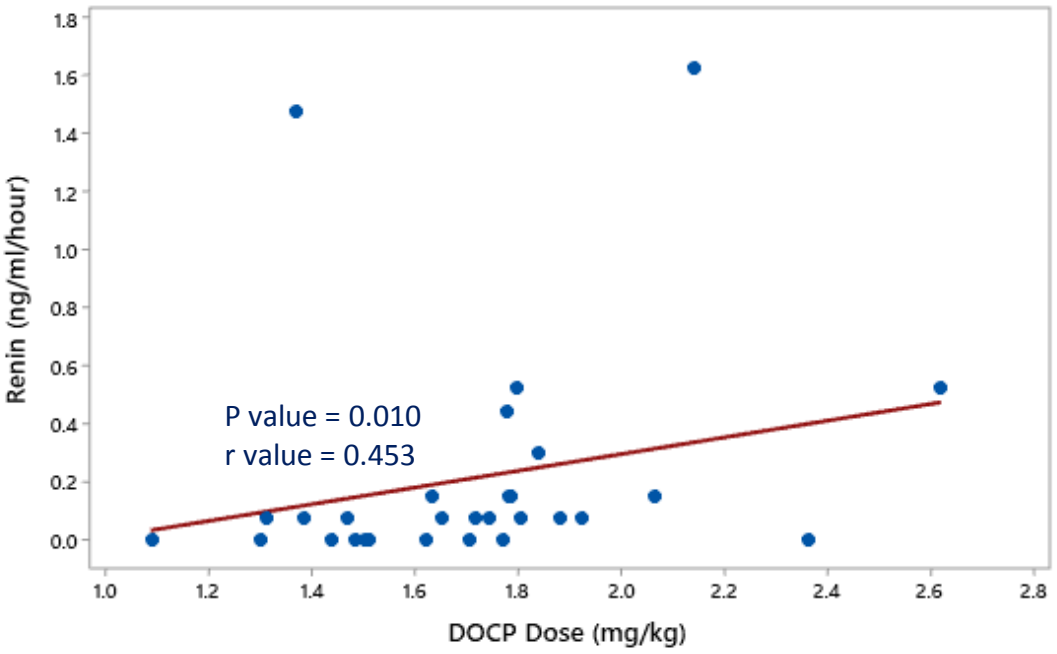
Box and whisker plot of the renin concentrations at the start and end of each phase of the trial. The coloured box represents the interquartile range (Q3 to Q1; 50% of the data set) with the solid bar within the box representing the median result (Q2). The whiskers show the remaining data (the 5th to the 95th percentiles). Outliers are marked by a symbol. Outliers are observations which are at least 1.5 times the interquartile range (Q3-Q1) from the edge of the box.



The renin concentrations were significantly lower at the end of the DOCP phase compared to the start (Wilcoxon Signed Rank test; P=0.000), but were not significantly different between the start and end of the fludrocortisone phase. They were also significantly lower at the end of the DOCP phase compared to the fludrocortisone phase (Wilcoxon Signed Rank test; P=0.000). Spearman correlation showed weak correlation (Figure 11) between the DOCP dose (mg/kg) and the renin concentration (P=0.010). This was not shown with fludrocortisone dose (mg/kg) and renin concentration.

Figure 11. Relationship between DOCP dose and renin at the end of the DOCP phase of the trial

Scatterplot of the relationship between the DOCP dose and the renin results. Spearman correlation showed positive correlation. The P value and r value are shown.



3.4.7.3 Blood pressure measurement

The blood pressure was measured at each visit and was evaluated alongside heart rate. The systolic blood pressure (mmHg) was found to be significantly higher at the end of the DOCP phase (mean 161mmHg; SD 26.3) compared to the fludrocortisone phase (mean 147mmHg; SD 26.2) (Paired T test; P=0.006; Paired) and also at the start and the end of the DOCP phase (Paired T test; P=0.001). There was no significant difference between the systolic blood pressure at the start and end of the fludrocortisone phase. Using Spearman Correlation, there was no correlation between the systolic blood pressure at the end of each phase of the trial and the renin concentrations, and between the systolic blood pressure and either the DOCP dose or fludrocortisone dose (mg/kg) at the end of each trial phase. Table 12 and Figures 12 to 16 summarise the blood pressure results from different points on the trial.

Assessing the cumulative scores of blood pressure from each trial visit during each phase of the trial (see section 2.7.3.3), the blood pressure decreased during the fludrocortisone phase and increased during the DOCP phase. The cumulative score at the end of the DOCP phase (24) was considerably higher than at the end of the fludrocortisone phase (11).

Table 12. Summary of blood pressure at each visit on each phase of the trial

This table shows the mean and standard deviation of the systolic blood pressure readings (mmHg) at each visit of the trial. The numbers in the pale blue boxes show the systolic blood pressure in mmHg.

	A	B	C	D	E
DOCP & Pred Phase					
Mean	154	148	141	147	148
Standard Deviation	22.1	26.8	26.2	22.0	26.3
Fludrocortisone Phase					
Mean	143	154	147	159	161
Standard Deviation	18.8	25.7	25.7	22.3	26.2
P Values					
Start and end of DOCP Phase					0.001*
Start and end of Fludrocortisone Phase					0.136
End of each Phase					0.006*

*Significant

Figure 12. Blood pressures at differential time points during the trial

Box and whisker plot of the blood pressure (systolic mmHg) at the start and end of each phase of the trial. The coloured box represents the interquartile range (Q3 to Q1; 50% of the data set) with the solid bar within the box representing the median result (Q2). The whiskers show the remaining data, the maximum to the minimum, with no outliers.

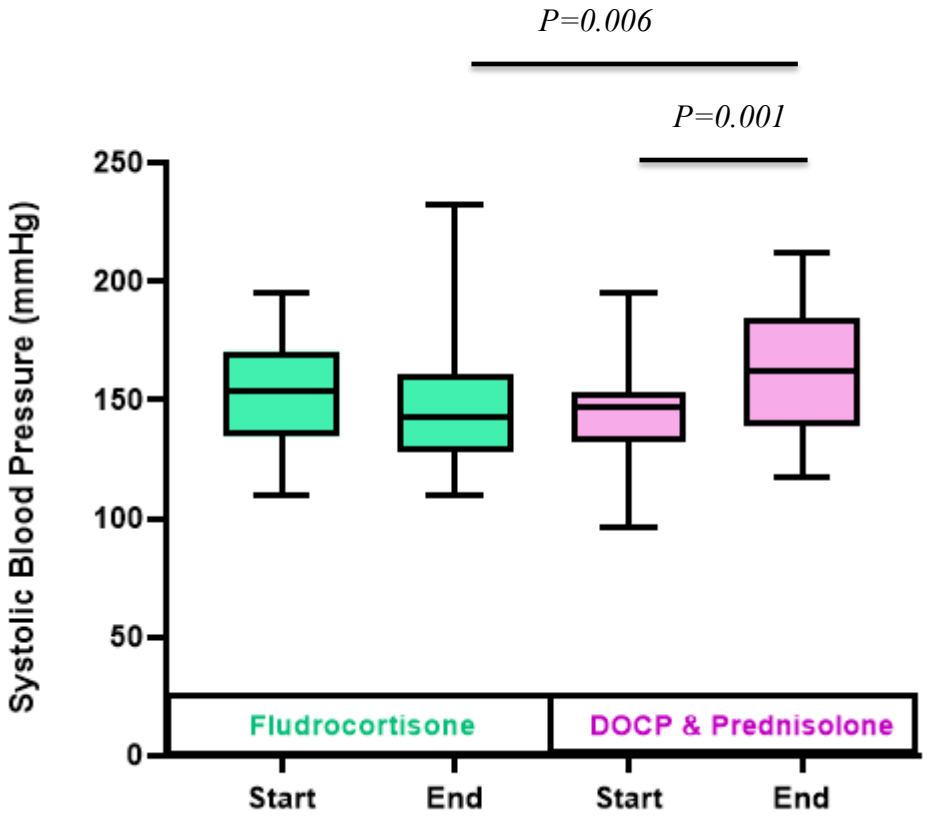


Figure 13. Comparison of mean systolic blood pressure on each phase of the trial

Line graph of the mean systolic blood pressure (mmHg) for all dogs on each visit during each phase of the trial.

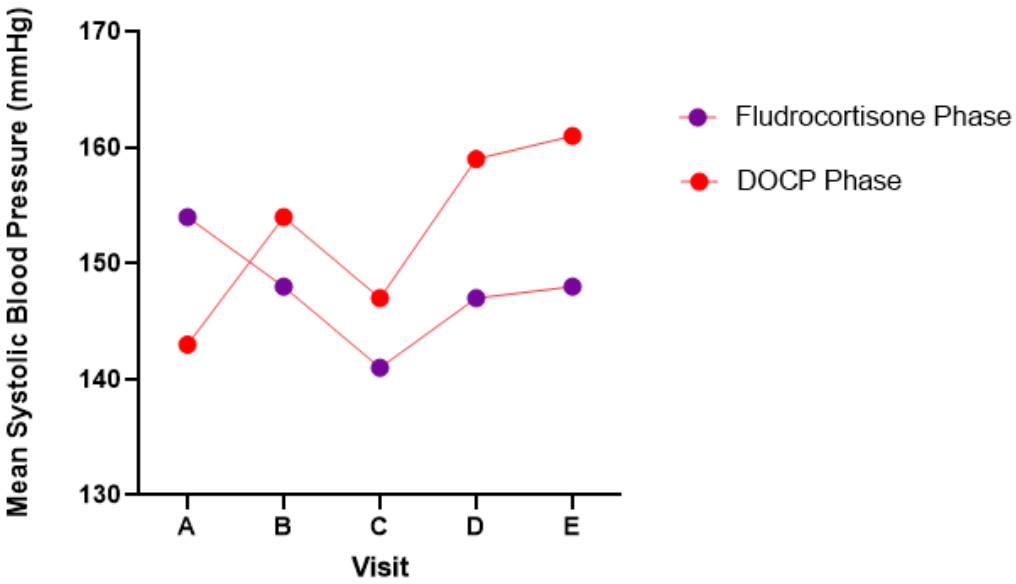


Figure 14. Relationship between systolic blood pressure and DOCP dose at the end of the DOCP Phase of the trial

Scatterplot of the systolic blood pressure of all patients at the end of the DOCP Phase of the trial (x axis) and their DOCP dose at this time (y axis).

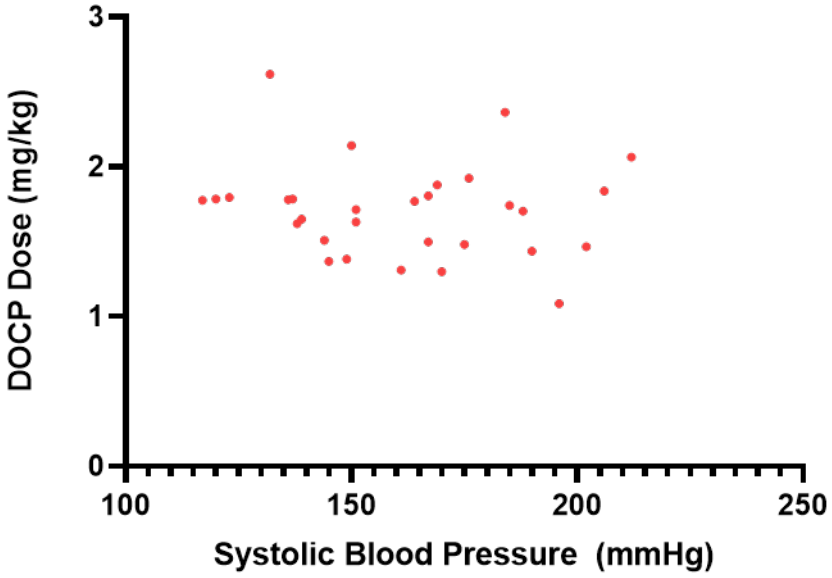


Figure 15. Relationship between systolic blood pressure and fludrocortisone dose at the end of the Fludrocortisone Phase of the trial

Scatterplot of the systolic blood pressure of all patients at the end of the Fludrocortisone Phase of the trial (x axis) and their fludrocortisone dose at this time (y axis).

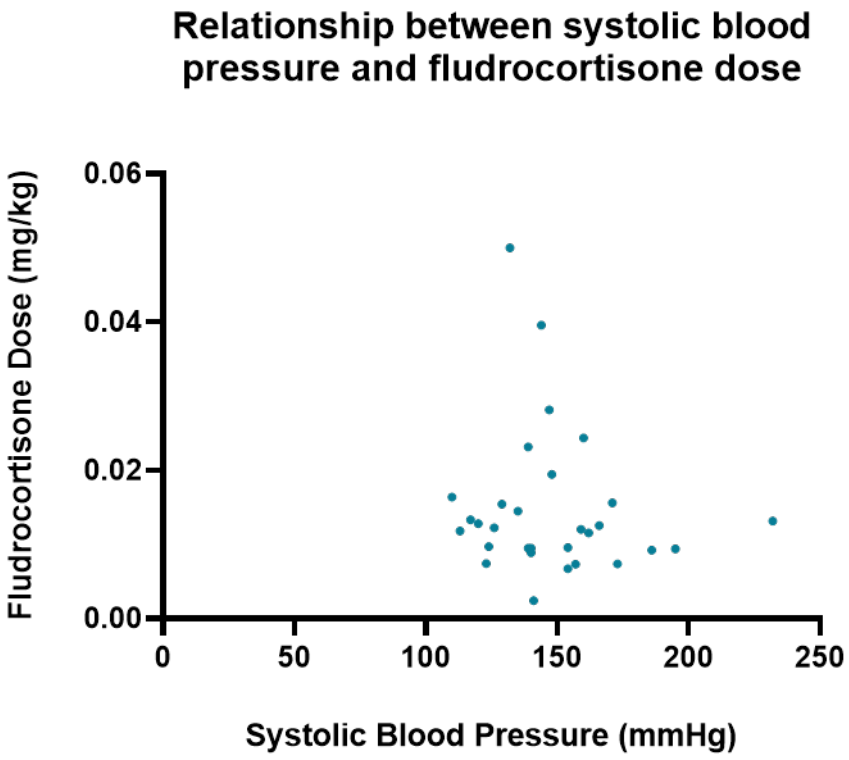
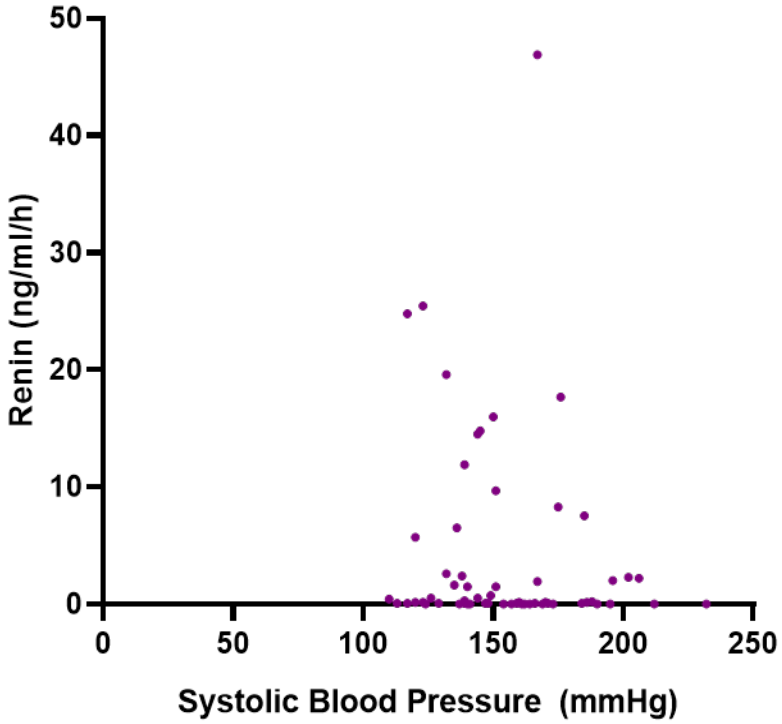


Figure 16. Relationship between systolic blood pressure and renin concentrations at the end of each phase of the trial

Scatterplot of the systolic blood pressure of all patients at the end of each phase of the trial (x axis) and their renin concentration at this time (y axis).



3.4.7.4 Haematology and biochemistry

The only significant haematological finding was an increase in the neutrophil count at the end of the DOCP phase (mean 7.49x10^9/L; SD 3.1) compared to the fludrocortisone phase (mean 5.81x10^9/L; SD 2.31) (Paired T Test; P= 0.009). There were no significant differences between the lymphocyte and eosinophil counts at the end of each phase of the trial using a Wilcoxon Signed Rank test. On biochemistry, the urea was significantly lower (P=0.000; Wilcoxon Signed Rank test) at the end of the DOCP phase (median 5.3mmol/L; 2.9-11.7) compared to the end of the fludrocortisone phase (median 7.4mmol/L; 3.8-17.2). The creatinine was also significantly lower (Paired T test; P=0.001) at the end of the DOCP phase (mean 96µmol/L; 19.2) compared to the fludrocortisone phase (mean 109µmol/L 28.9). There was no significant difference in the albumin (Wilcoxon Signed Rank test), glucose (Wilcoxon Signed Rank test) or calcium (Paired T test) concentrations at the end of the DOCP phase compared to the fludrocortisone phase. Table 13 summarises these results.

Table 13. Summary of haematology and biochemistry results

The range and median of the main haematological and biochemical results. Although full haematology and biochemistry analytes were performed, these were considered to be the parameters directly associated with hypoadrenocorticism and its treatment. Therefore statistical analyses were performed only on these results.

Variable	Phase	Minimum	Maximum	Median	P value
Neutrophils	DOCP & Pred	0.48	14.23	7.51	0.009*
	Fludrocortisone	0.47	9.57	6.48	
Lymphocytes	DOCP & Pred	0.29	1.99	1.12	0.119
	Fludrocortisone	0.25	4.29	1.05	
Eosinophils	DOCP & Pred	0.00	0.74	0.18	0.581
	Fludrocortisone	0.00	1.22	0.18	
Urea	DOCP & Pred	2.9	11.7	5.3	0.000*
	Fludrocortisone	3.8	17.2	7.4	
Creatinine	DOCP & Pred	63	146	97	0.001*
	Fludrocortisone	65	192	107	
Albumin	DOCP & Pred	28	44	33	0.209
	Fludrocortisone	25	41	33	
Glucose	DOCP & Pred	0.0	9.1	5.0	0.509
	Fludrocortisone	4.0	6.1	4.9	
Calcium	DOCP & Pred	2.31	3.09	2.63	0.705
	Fludrocortisone	2.27	3.26	2.63	

*Significant

3.4.8 Non-inferiority

No dogs were hyperkalaemic at the end of the fludrocortisone or DOCP phases of the trial however three dogs were hyponatraemic at the end of the fludrocortisone phase. When using signs of glucocorticoid and mineralocorticoid under dose only, no dogs had clinical signs at the end of either phase of the trial. Using these factors as indicators of success (lack of hyperkalaemia or hyponatraemia and no clinical signs of hypoadrenocorticism), DOCP and prednisolone were found to be non-inferior to fludrocortisone for the management of hypoadrenocorticism in dogs (Table 14). This is as the success in the DOCP and prednisolone group was 100% and in the fludrocortisone was 90%. The lower bound 95% confidence interval of this difference (10%) was -1% and this was within the set non-inferiority margin of 22%.

Table 14. Summary of Non-inferiority calculation

This table shows how non-inferiority is calculated. It compares the primary outcome measures i.e. clinical signs and electrolytes. Only electrolyte results suggestive of under treatment (i.e. hyperkalaemia or hyponatraemia) were used. For DOCP and prednisolone to be non-inferior to fludrocortisone, the lower bound of the two-sided 95% confidence interval of the difference had to be less than 20% than that of fludrocortisone.

	N per group	% CP no hyperkalaemia or hyponatraemia or no CS	% IVP no hyperkalaemia or hyponatraemia or no CS	% CP - IVP	Lower bound of the two-sided 95% confidence interval of the difference	Decision Outcome
Na	**30**	**90%**	**100%**	**-10%**	**-1%**	**Non-inferior**
K	**30**	**100%**	**100%**	**0%**	**0**	**Non-inferior**
CS overdose	**30**	**17%**	**17%**	**0%**	**0**	**Non-inferior**
CS under dose	**30**	**100%**	**100%**	**0%**	**0**	**Non-inferior**
Success if use CS overdose	**30**	**13%**	**17%**	**-4%**	**-12%**	**Non-inferior**
Success if use CS under dose	**30**	**90%**	**100%**	**-10%**	**-1%**	**Non-inferior**

Key

CS – Clinical signs

CP – Control product; fludrocortisone phase

IVP – Interventional product; DOCP and prednisolone phase

CS overdose = increased thirst, increased urination, increased blood pressure, lethargy, coat changes, polyphagia

CS underdose = vomiting, diarrhoea, lethargy

(results of overdose and underdose were considered separately as only clinical signs of underdose were considered to be significant when considering drug efficacy)

4 Discussion

4.1 Study Design

4.1.1 General Study Design

This study showed that DOCP and prednisolone were not inferior when compared to fludrocortisone acetate in the management of canine hypoadrenocorticism. Although several studies have shown the success of DOCP (Percorten®) (Kintzer and Peterson, 1997b, Lynn and Feldman, 1991, Lynn et al., 1993, Melian and Peterson, 1996) in the management of this condition, the drug has never been directly compared to fludrocortisone acetate. The initial studies simply demonstrated efficacy with no comparison (Lynn and Feldman, 1991, Lynn et al., 1993). Later studies examined dogs with hypoadrenocorticism that had been transitioned from one drug to another when they have not been considered to be stable (Kintzer and Peterson, 1997b, Melian and Peterson, 1996). More recently, Zycortal® has been shown to be non-inferior to Percorten® however treatment with DOCP was not compared to fludrocortisone with prednisolone (Farr et al., 2020).

In this study, a cross over design was used to compare DOCP and prednisolone to fludrocortisone acetate, with a cohort of patients receiving both drugs in a random order whilst being monitored in as uniformly as possible. Randomisation into two groups was necessary to avoid sequence bias when comparing treatments. A single study site was selected to minimise the number of clinicians involved in the trial and to ensure consistency in dose adjustments which were unavoidably subjective in nature. Regular meetings were arranged to discuss dosing decisions. This was important when considering the presence of clinical signs which was critical for assessment of glucocorticoid supplementation as no objective measure is readily available. In addition, this also allowed comparisons between laboratory data to be made using the same analysers.

4.1.2 Non-inferiority

A non-inferiority study design was selected over a superiority study design as hypoadrenocorticism is an uncommon condition and the case numbers required for a superiority study were considered to be unattainable. Non-inferiority was considered to be an adequate assessment of clinical control as studies have already shown fludrocortisone to provide adequate control of hypoadrenocorticism (Freise et al., 2013, Huitfeldt et al., 2011, Roberts et al., 2016). Superiority was therefore not required and instead it was simply necessary to determine that the licensed product was non-inferior to the unlicensed product.

A cross-over design was chosen over a placebo-controlled trial as it was considered to be more practical. One way of performing a placebo-controlled trial would be to give every dog both an injection and tablets – one a placebo and one with an active ingredient. This was considered impractical, as clinicians were required to be aware which drug was being administered to assist with dose adjustments during the trial.

4.1.3 Outcome Measures

The outcome measures were considered to be primary or secondary, where the primary measures were used to directly consider treatment success and secondary outcomes were retrospectively used as an adjunctive measure of drug efficacy. Primary outcome measures were electrolyte results and clinical signs. Electrolytes were chosen as they are a direct and rapid measure of mineralocorticoid supplementation which can be used to make adjustments to the DOCP dose. Clinical signs were considered to be the most practical and reliable measure of glucocorticoid overdose. Although endogenous ACTH might provide a reflection of the adequacy of glucocorticoid supplementation, this has not been formally demonstrated and, furthermore, it relies on measurement of a labile hormone at a specialist endocrine laboratory. Secondary outcome measures were not used to assess non-inferiorty however they were used to provide an overall assessment of clinical control of hypoadrenocorticism. Several secondary measures were used. The most useful of these were felt to be the measurement of endogenous ACTH and renin, which should reflect glucocorticoid and mineralocorticoid supplementation respectively. These results were reviewed retrospectively and were not used to assist with dose adjustments. Aldosterone is integral to the renin-angiotensin-aldosterone system which controls circulating volume and effectively, blood pressure. In addition, cortisol also exerts some effect on blood pressure. Therefore blood pressure was considered to be a useful secondary measure of control. Routine haematology and biochemistry were used as secondary measures to assess glucocorticoid and mineralocorticoid supplementation with a neutrophilia and monocytosis being associated with glucocorticoid overdose. A high urea and creatinine was thought to suggest mineralocorticoid underdose and hypoalbuminaemia or hypoglycaemia being associated with glucocorticoid underdose.

4.1.4 Protocol Deviations

Following the start of the trial, several amendments of the study protocol were required. These were to ensure completion of the trial with minimal bias and limitations.

Initially the study protocol had stated that non-inferiority would be determined by treatment success at each visit during each phase of the trial (section 2.1). However it quickly became evident that patients were requiring several dose adjustments before being considered stable on DOCP and therefore, the treatment success at the end of each phase of the trial was considered for non-inferiority instead.

In addition, it became evident during the study that clinical signs of glucocorticoid overdose and hypokalaemia/hypernatraemia suggestive of mineralocorticoid overdose were common during the DOCP phase (section 3.3.6). These results suggested over supplementation of DOCP and prednisolone for management of hypoadrenocorticism and using the initial classification of non-inferiority, this treatment would have been found to be non-inferior to fludrocortisone. However, as the aim of the study was to ensure that patients were not being under supplemented with medication for management of hypoadrenocorticism, the non-inferiority criteria were adjusted to consider only patients with signs of underdosing.

During the study period the branded preparation of fludrocortisone acetate (Florinef®, Bristol) became unavailable. Therefore a generic fludrocortisone tablet formulation (Aspen Pharmaceuticals) was used instead. This formulation was more expensive and only limited stocks were available (even for human use). Therefore the protocol was re-appraised to reduce the use of fludrocortisone. It was noted that the washout period at the end of the fludrocortisone phase was not needed and so this was stopped. As a result only one dog had a washout period when treatment had been started with fludrocortisone. This meant the total trial period varied between the two groups of patients but this was not considered to affect the study results. The generic tablets did not require storing at refrigerated temperatures however their pharmacokinetics were assumed to be the same as Florinef®. This is a potential limitation of the study.

4.1.5 Visits

Visit compliance throughout the study was good (section 3.3.3, Table 8 and Figure 2). This was most likely due to careful client education prior to patient enrolment, to ensure willingness to comply. Some visits were slightly early or late however efforts were made to ensure the visits were within 3 days of their scheduled date to allow reliable comparison between patients. Due to unavoidable issues such as owner illness or work commitments, some visits were outwith this window.

The number of unscheduled visits was far greater than expected. The majority of these visits were during the DOCP phase of the trial. The reasons for this were mainly due to the starting dose of DOCP being too high in most patients and numerous dose reductions being required. This meant that 10 day checks were required for many patients during the DOCP phase of the trial (section 3.3.4). The trial protocol has accounted for ten day visits (B visits) ten days after the A visit. If the starting dose of DOCP was lower, the majority of these visits would not have been required. In addition, there were a significant number of unscheduled visits required as DOCP injections were having a prolonged duration of action (most likely due to the high starting dose), or less commonly, a short duration of action. As discussed, patients 13 had several unscheduled visits due to suspected adverse effects of DOCP (section 3.2).

4.2 Study Population

4.2.1 Inclusion and Exclusion Criteria

Three patients were excluded from the trial: one was not diagnosed with an ACTH stimulation test, one was diagnosed with hypothyroidism at enrolment and one died of a ruptured haemangiosarcoma.

There were two patients who did not meet the initial inclusion/exclusion criteria but that were included in the trial. Patient 29 was included in the trial despite being on topical steroid eye drops. The dose of this medication was unchanged during the trial period and the route of administration of this drug was topical rather than systemic. This patient had also been on eye drops containing a glucocorticoid for 13 days before its ACTH stimulation test. Although adrenocortical suppression has been suggested with ophthalmic steroid preparations (Roberts et al., 1984, Zenoble and Kemppainen, 1987), it is more widely accepted to be associated with otic steroid preparations (Ghubash et al., 2004). As topical steroid preparations have been shown to reduce the responsiveness of cortisol to ACTH stimulation rather than causing clinical adrenocortical failure (such as electrolyte derangements and gastrointestinal signs), this patient was not excluded from this study. The patient did have electrolyte derangements (hyperkalaemia and hyponatraemia) at the time of diagnosis. In addition, the patient responded favourably to mineralocorticoid and glucocorticoid supplementation and had a signalment which was consistent with primary spontaneous hypoadrenocorticism. Considering these factors, this patient was included in the trial.

One patient (Patient 15) had a pre-existing diagnosis of a recurrent malassezia otitis externa. This had resolved by the time of enrolment however it had been treated with oral ketoconazole and amoxicillin clavulanic-acid (Synulox, Zoetis). The patient had become unwell eight days following the introduction of ketoconazole, at which point this drug had been discontinued and ACTH stimulation test was performed to investigate hypoadrenocorticism as a cause of illness. Although ketoconazole is used to treat hyperadrenocorticism and causes inhibition of multiple cytochrome P450 enzymes including those involved in steroidogenesis (Sanders et al., 2018), it has not been reported to show results of ACTH stimulation tests which mimic that of primary hypoadrenocorticism. Therefore, although ketoconazole can result in a reduced response to an ACTH stimulation test, it is not classically considered to cause a complete lack of stimulation as is often seen with hypoadrenocorticism (Lathan and Thompson, 2018) and this patient was not excluded from this study.

4.2.2 Patient Illness During The Study Period

Three patients required antibiosis for management of pyoderma and one patient for a urinary tract infection. The conditions and the antibiotics used were not considered to interfere with the trial protocol. These conditions were not considered to be an adverse reaction to the trial drugs. However, it is possible that chronic steroid therapy, particularly if at doses higher than required for glucocorticoid replacement, could lead to an increased risk of development of infections due to suppression of the immune system.

One patient was prescribed clindamycin by its primary care veterinarian to manage an episode of colitis. Although this drug was not thought to interfere with the trial, it is possible that the colitis could have been considered a sign of hypoadrenocorticism. As this diarrhoea responded to antibiotic therapy and as there were no other signs fitting with unstable hypoadrenocorticism, it was suspected to be an unrelated event.

Several other drugs including analgesics and urinary incontinence drugs were prescribed during the trial however these were not considered to interfere with the trial protocol. Two patients did require management for suspected trial drug related events.

Patient 13 required recurrent hospitalization and unscheduled visits. These ranged from 5 to 16 days post DOCP injection and two unscheduled visits were required each time. Supportive treatment was required for signs of nausea, lethargy and weakness. During these episodes this patient was hypokalaemic and hypertensive. In addition, each episode occurred at around day 10 following DOCP injections. Therefore, these events were suspected to be due to mineralocorticoid over-supplementation. Spironolactone was given as an aldosterone antagonist to resolve these effects of mineralocorticoid overdose. On one visit, buprenorphine was also prescribed as the patient had marked abdominal discomfort. This patient was suspected to have pancreatitis as a point of care analyser for canine specific pancreatic lipase (SNAP cPL^{TM,} IDEXX) was abnormal and there were also ultrasonographic changes supportive of this condition. Although the onset of pancreatitis could have been unrelated to the trial, it is possible that this was a side effect of the DOCP given the condition occurred when the drug was at its highest systemic concentration. This patient did not require any unscheduled visits during the fludrocortisone phase of the trial, nor did it show any abnormal clinical signs during this phase.

Patient 22 received maropitant 9 days after its third dose of DOCP due to vomiting and the onset of signs of nausea (lip smacking and inappetence). Only one dose of buprenorphine was required and it is likely that this was an incidental sign. However given this occurred 9 days after DOCP injection, at the time when the drug is suspected to be at its highest concentration, it has to be considered that this event could have been drug related. More specifically, as another patient suffered recurrent pancreatitis as discussed, it is possible that this was the cause of the vomiting. Further investigations were not performed given the quick resolution of the signs.

There seems to be no theoretical link between pancreatitis and over supplementation of mineralocorticoid. Patients with naturally occurring mineralocorticoid excess (such as feline primary hyperaldosteronism in veterinary medicine) do not commonly develop pancreatitis (Andrew et al., 2005, Djajadiningrat-Laanen et al., 2011). Glucocorticoid excess has been speculated to be associated with pancreatitis and hyperadrenocorticism is thought to be a risk factor for the development of acute pancreatitis (Hess et al., 1999). Other dogs with hyperadenocorticism show increased Spec cPLI without clinical signs of pancreatitis (Mawby et al., 2014). However, although exogenous glucocorticoids are expected to increase the release of pancreatic enzymes from the pancreas, clinical pancreatitis has not been demonstrated experimentally (Parent, 1982). Several studies have looked at the development of pancreatitis with exogenous glucocorticoid supplementation and have not shown the same link to pancreatitis as with hyperadrenocorticism (Barzilai et al., 1986, Fittschen and Bellamy, 1984). The mechanism of the potential increased incidence of pancreatitis with excessive glucocorticoid concentrations is unknown and it is possible that a similar mechanism is responsible for dogs developing pancreatitis when being treated with DOCP and prednisolone. As the suspected episodes of pancreatitis developed at the point of maximum mineralocorticoid concentrations, it seems more likely that it is related to mineralocorticoid overdose. This should be considered when treating patients with DOCP and further studies are required to investigate the pathological mechanism involved.

4.3 Study Results

4.3.1 Study Population

There were 16 dogs in Group A and 14 dogs in Group B. The discrepancy between group numbers was due to 33 doings being enrolled and three being excluded; two dogs excluded were in group B and only one was group A. The general study population was representative of dogs with hypoadrenocorticism. The weight of the two groups was statistically significantly different however this was not suspected to be of clinical relevance as all doses were calculated in mg/kg. The gender distribution was also different between the groups but as no studies have shown any differences in disease severity or response to treatment between gender or neuter status, this was also not considered to be significant. Therefore, the two groups were combined for analysis and each dog acted as its own control given the cross-over design.

4.3.2 Indicators of Disease Control (Outcome Measures)

4.3.2.1 Electrolytes

The primary outcome measure for assessing mineralocorticoid control was the sodium and potassium concentrations. Either hypokalaemia or hypernatraemia was considered to indicate over supplementation of mineralocorticoid and conversely, hyperkalaemia and hyponatraemia indicated under supplementation. Although concurrent illness or laboratory analyser error could have both led to electrolyte abnormalities, the assumption was made that any electrolyte derangements were related to mineralocorticoid supplementation. This was as there was no method of controlling this and patients did not show clinical signs to suggest otherwise.

Although dogs enrolled on the trial were considered to be stable on fludrocortisone treatment, the sodium concentrations were below the lower reference range 5 times during the fludrocortisone phase of the trial. This would suggest under supplementation of mineralocorticoid. It is however important to note that the lowest sodium result of the trial was at the E visit of the fludrocortisone phase of the trial and that this result was considered to be spurious (111.8 mmol/L) because such a marked hyponatraemia would be expected to be associated with clinical signs. In addition, the sodium was measured on a point-of-care machine from the same sample and was found to be 147 mmol/L.

One patient had low potassium whilst on the fludrocortisone phase of the trial. No hypokalaemia had been noted previously and it resolved on subsequent visits without a fludrocortisone dose reduction. Therefore, this result was also considered to be spurious.

In contrast, the potassium concentrations were low on 12 occasions during the DOCP phase trial. This is suggestive of mineralocorticoid excess and given this finding was not seen on the fludrocortisone phase, it would suggest overdosing of DOCP. No patients had hyperkalaemia or hyponatraemia to suggest under supplementation of mineralocorticoid during the DOCP phase of the trial.

Comparing the two phases of the trial, both the sodium and potassium concentrations at the end of each phase of the trial were significantly different (section 3.3.6.1). During the DOCP phase, the potassium concentrations generally decreased and the sodium concentrations increased. The opposite was true for fludrocortisone, suggesting fludrocortisone was more commonly associated with under supplementation of mineralocorticoid and DOCP with over supplementation. Patients were considered to be stable on fludrocortisone at the time of enrolment and therefore this was an unexpected finding of the trial.

The DOCP dose (mg/kg) showed weak positive correlation with the potassium concentrations at the end of the DOCP phase of the trial. This finding was not considered to be significant as it was clearly observed that reducing DOCP dose increased the potassium levels during the study. As this correlation was between the DOCP dose at the end of the DOCP phase and the potassium levels, it may not be representative of the relationship throughout the trial.

Using the target range of 27-32 for the sodium:potassium ratio, many dogs were perceived to be well controlled clinically but with high sodium:potassium ratios. Therefore this range provides an inaccurate and overly sensitive method for monitoring hypoadrenocorticism. It is concluded that 32 seems to be too low to be suitable for the upper reference range for the sodium:potassium ratio when monitoring dogs on DOCP, as this would have incorrectly led to dose reductions in dogs with individual electrolytes within the normal reference range. Increasing the upper reference range to 42 would be more clinically appropriate for detecting over treatment with DOCP. Conversely, using the sodium:potassium ratio missed dogs with hyponatraemia that were being under supplemented with fludrocortisone. Therefore, a more appropriate approach may be to consider each electrolyte result individually, with respect to its own reference range, instead of following the guidance supplied by the manufacturer (Table 3).

4.3.2.2 Clinical Signs

Despite the electrolyte concentrations being suggestive of under supplementation of mineralocorticoid in dogs during the fludrocortisone phase of the trial, no dogs showed clinical signs of mineralocorticoid or glucocorticoid deficiency during this phase. One dog (Patient 13) did show signs which could be consistent with mineralocorticoid or glucocorticoid deficiency during the DOCP phase of the trial, however given concurrent hypokalaemia and hypertension and considering the timing of this episodes, these signs were instead suspected to be due to mineralocorticoid over supplementation.

During the fludrocortisone phase of the trial, the number of dogs showing clinical signs of glucocorticoid excess decreased. This may have been as group A dogs were on prednisolone with DOCP prior to starting the fludrocortisone phase. This was in contrast to the DOCP phase where the clinical signs of glucocorticoid excess increased substantially. During the DOCP phase, prednisolone was given concurrently with DOCP. Most dogs required a dose reduction of both drugs and overall it seems that both glucocorticoids and mineralocorticoids were being over-supplemented in many dogs during this phase of the trial.

It was very difficult to use clinical signs of mineralocorticoid excess such as polyuria, polyphagia and weakness as markers of control as they were non-specific signs and could be consistent with either mineralocorticoid or glucocorticoid deficiency or excess. Day 10 was thought to be the most fitting time for signs of mineralocorticoid excess to manifest as this is when DOCP is thought to be at its highest systemic concentration. Generally though, blood pressure and weakness were assessed to give a more specific marker of mineralocorticoid control. Looking at blood pressure, the systolic blood pressure was significantly higher during the DOCP phase compared to the fludrocortisone phase of the trial. This again fits with the suggestion that DOCP is a very efficacious drug and the starting dose is too high for the majority of patients.

4.3.2.3 ACTH concentrations

ACTH is produced from the pituitary gland and increases cortisol (and to a lesser extent aldosterone) production from the adrenal gland, however dog to dog variation may exist. Negative feedback exerted on the pituitary gland by cortisol, leads to reduction of ACTH production. Therefore, if endogenous or exogenous glucocorticoid concentrations are high, ACTH production can be suppressed. It was hypothesised that endogenous ACTH might be a useful marker of glucocorticoid control, with ACTH being high with glucocorticoid deficiency and low with glucocorticoid excess.

ACTH concentrations were reduced in over two thirds of dogs in the DOCP phase of the trial. The concentrations were actually undetectable in half of the dogs during the DOCP phase of the trial. This is consistent with the clinical signs of glucocorticoid overdose and suggests that the prednisolone doses used were too high. There was however no correlation between prednisolone dose (mg/kg) and the ACTH concentrations. This fits with observations that the glucocorticoid supplementation required is very variable between individual patients.

4.3.2.4 Plasma renin activity

Renin is produced from the juxtaglomerular apparatus of the kidneys in response to low circulating blood volume and high sodium concentrations. It leads to the activation of the RAAS system (including aldosterone release from the adrenal gland) and subsequently to water reabsorption from the kidneys. Therefore, excessive aldosterone concentrations would be expected to cause suppression of plasma renin activity and mineralocorticoid deficiency would cause an increase in plasma renin activity. It was hypothesised that renin concentrations may be a useful marker for mineralocorticoid control.

During the DOCP phase, the renin concentrations decreased in the majority of dogs. In addition, the renin concentrations were almost undetectable in almost two thirds of the patients during the DOCP phase of the trial. The renin concentrations were significantly lower at the end of the DOCP phase of the trial compared to the fludrocortisone phase. There was significant correlation between the DOCP dose (mg/kg) and the renin concentrations. This suggests the DOCP has potent mineralocorticoid actions and particularly in patients where the renin activity was completely undetectable, perhaps the DOCP dose was too high. It is possible that dogs with undetectable renin concentrations would have had normalization of plasma renin activity with further dose reductions of DOCP, however follow-up samples were not obtained after conclusion of the trial.

There was no appreciable relationship between the fludrocortisone dose (mg/kg) and the renin concentrations. In several patients being treated with fludrocortisone the renin increased and was above the upper reference limit. As discussed, these patients showed no signs of mineralocorticoid deficiency. This raises concerns that patients being treated with fludrocortisone could be being under-supplemented with mineralocorticoid, despite showing no clinical signs of hypoadrenocorticism.

4.3.2.5 Blood pressure measurement

Blood pressure was used as an indirect marker of mineralocorticoid control, with normotension being consistent with good control, hypotension being consistent with under control and hypertension being consistent with over control. The systolic blood pressure (mmHg) was found to be significantly higher at the end of the DOCP phase compared to the fludrocortisone phase and also at the start and the end of the DOCP phase. This is another factor which is supportive of DOCP efficacy and effective mineralocorticoid supplementation. Despite this finding, there was no relationship found between the DOCP dose and the blood pressure or the renin concentrations and the blood pressure. Although the renin concentrations were high in several patients on the fludrocortisone phase of the trial, no patients were hypotensive.

4.3.2.6 Haematology and biochemistry

The only significant haematological finding was an increase in the neutrophil count at the end of the DOCP phase compared to the fludrocortisone phase. This is most likely due to prednisolone therapy during this phase of the trial, with glucocorticoids (endogenous and exogenous) leading to a neutrophilia or a "stress leukogram". This could again be suggestive of glucocorticoid over supplementation during the DOCP phase of the trial.

On biochemistry, the urea and creatinine concentrations were both significantly lower at the end of the DOCP phase compared to end of the fludrocortisone phase. Although prednisolone therapy can cause polyuria with subsequent decreases in urea, this could also be consistent with mineralocorticoid excess. These results suggest adequate management of hypoadrenocorticism as poorly managed patients trend towards azotaemia. Otherwise there were no statistical differences on biochemistry between the two groups.

4.3.2.7 Overall Interpretation of Outcome Measures

These results would suggest that during the DOCP phase of the trial, both mineralocorticoids and glucocorticoids were over supplemented and further dose reductions should have been considered. Given the trial protocol, further drug adjustments could not be achieved during the trial. However following completion of this trial, several dogs required and benefited from further dose reductions. Despite this, all dogs were perceived to be clinically well controlled at the end of the trial.

In contrast, there was suggestion that dogs treated with fludrocortisone were undertreated with mineralocorticoids and glucocorticoids, based on endogenous ACTH and plasma renin activity result.

4.3.2.8 Statistical analysis

Although numerous statistical tests including Paired T tests, Wilcoxon Signed Rank tests and Spearman/Pearson correlation were performed (section 2.7), each individual variable was not tested more than twice, or three time in the case of blood pressure analysis. Despite this, Bonferroni adjustments were made to all P values to assess how this would affect the study results. Of the 33 statistical tests with P value corrections, the significance was affected on only one occasion. In this instance, the number of patients with hypertension and weakness was significantly different at the end of the fludrocortisone phase compared to the end of the DOCP phase, with a P value of 0.035 when using a cut off of P= 0.05. However, with Bonferroni correction the P value was adjusted to 0.025 which meant the P value of 0.035 lost significance.

The Bonferroni-Holm method is a less conservative method of adjusting the P value to reduce the risk of getting type I errors (ie finding significance due to chance given the number of statistical tests performed). It is possible that this method of P value adjustment could have led to a greater proportion of results losing significance, however this method was not performed. This was for several reasons such as: avoidance of a type I error was not considered more important than avoidance of a type II error; although numerous statistical tests were performed, they were not performed repetitively on the same result; the study was prospective and the statistical tests used were pre-determined and finally, these adjustments in P values would have effected only the secondary and not the primary outcome measures and therefore would not have affected the non-inferiority calculation (Armstrong, 2014).

4.3.3 Drug Doses and Dose Adjustments

Overall, there were very few fludrocortisone dose adjustments required as patients were considered stable at the point of entry. Dose reductions were required due to clinical signs of glucocorticoid overdose. There were no apparent clinical signs or biochemical changes consistent with mineralocorticoid overdose or under dose during the fludrocortisone phase. Dogs enrolled on the trial were perceived to be clinically stable on fludrocortisone and therefore it is not surprising that dose adjustments were uncommon. As fludrocortisone has both mineralocorticoid and glucocorticoid activity, it can be challenging to find the correct dose for both glucocorticoids and mineralocorticoids concurrently, as dogs require different proportions of each steroid. The final median dose of fludrocortisone (0.012mg/kg twice daily) was similar to that recommended by the BSAVA Small Animal Formulary (Ramsey, 2017).

Conversely, DOCP was a new treatment for all dogs on the trial. Although DOCP has been used in the management of canine hypoadrenocorticism previously, Zycortal® had not been extensively used before. Therefore, starting doses were extrapolated from studies using a different drug formulation (Percorten®) and historical efficacy studies (Farr et al., 2020). It is therefore understandable that numerous dose adjustments were required throughout the trial period. Most commonly, dose reductions in DOCP were required. Numerous patients showed electrolyte derangements (most commonly hypokalaemia) and one patient (Patient 13) may have suffered recurrent illness due to the effect of mineralocorticoid excess.

Although a much lower starting dose seems advisable (median dose range at the end of the DOCP phase 1.74mg/kg every 28 days), it is important to note that two dogs required dosing every 25 days due to apparent reduced length of action of DOCP and one dog required dose increases to 2.6mg/kg every 28 days. As the clinical signs of under dosing of mineralocorticoid supplementation can be more dangerous and debilitating than over dosing, it seems sensible to start with a slightly higher dose with subsequent dose reductions than to start with a lower dose and require dose increases.

Over two thirds of dogs required prednisolone dose reductions during the DOCP phase of the trial. Only one dog was supplemented with prednisolone during any point of the fludrocortisone phase of the trial and this drug was discontinued during this phase. Dose reductions were advised due to the presence of a combination of polyuria, polydipsia, panting, polyphagia and coat changes. The former three of these signs can also be seen with mineralocorticoid excess and therefore this made interpretation of prednisolone dosage difficult.

Although the starting dose of prednisolone generally seemed too high, the final dose varied dramatically between individuals. There appeared to be no relationship between weight and prednisolone dose. One 56kg patient required only 1mg daily whereas a 20kg dog required 10mg of prednisolone daily. Therefore, it is important that thorough histories are obtained to ensure optimal prednisolone dosing and to avoid over or under supplementation of glucocorticoid. Both under and overdosing of prednisolone can have significant detrimental effects on patients.

The median dose of prednisolone at the end of the DOCP phase of the trial was 0.13mg/kg once a day (range 0.02-0.35). Although more commonly employed when giving larger doses of prednisolone, metabolic weight (e.g. mg/m^2 dosing) can be used as an alternative to mg/kg doses. This is thought to be more accurate and tends to lead to smaller mg/kg doses being used in larger dogs (Nam et al., 2017). In this study we did not evaluate the relationship between mg/m^2 dosing and different variables or look for significance of doses of prednisolone compared to weight when using mg/m^2 dosing (or other measures of metabolic weight). There have been no studies looking at mg/m^2 dosing with DOCP however this could be a more reliable way to dose dogs with DOCP given the vast range in doses required.

4.3.4 Overall Assessment of Treatment Response

This study showed that DOCP and prednisolone were non-inferior to fludrocortisone for the treatment of hypoadrenocorticism. This conclusion is based on the fact that no patients were hyponatraemic, hyperkalaemic or had clinical signs of hypoadrenocorticism at the end of the DOCP phase of the trial (and was confirmed using the non-inferiority calculation).

Therefore, this treatment could not be inferior to fludrocortisone, regardless of the results during the fludrocortisone phase. Three patients were hyponatraemic at the end of the fludrocortisone phase of the trial and this was indicative of an inferior performance of this drug compared to DOCP.

Two protocol amendments were required to reach this conclusion. This was as first of all, the starting dose of DOCP appeared to be too high with some patients requiring significant dose reductions. If adhering to the original study protocol (section 2.1) which stated that electrolytes must be within the normal reference range to consider "treatment success", then numerous treatments would have been considered unsuccessful due to over treatment. However clinically, this was not considered to be a treatment failure as with subsequent dose reductions following conclusion of the trial period, the patients with electrolytes out with the normal reference range at the end of the DOCP phase were later considered to be very well controlled. In addition, if considering the clinical signs and electrolytes at each visit, DOCP would have likely seemed inferior to fludrocortisone. The trial period was three months to allow for dose adjustments and stabilization on a new medication and therefore it was decided that the clinical signs and electrolyte results at the end of each phase of the trial was a more accurate reflection of control of disease.

At no points during the DOCP phase of the trial did patients show hyperkalaemia or hyponatraemia, which would fit with poor mineralocorticoid control. Although one patient was hyperkalaemic on the A visit of this phase, this would have been before DOCP was given and therefore reflective of mineralocorticoid supplementation from fludrocortisone. In addition, five dogs were hyponatraemic during the fludrocortisone phase of the trial, suggesting that mineralocorticoid supplementation was more likely to be inadequate during this treatment phase.

4.3.5 Follow Up

After conclusion of the trial, the majority of dogs returned to our hospital until considered stable on DOCP. All dogs were eventually discharged from our hospital with no signs of steroid deficiency or excess and with electrolytes within the normal reference range. Owners, who had been filling out quality of life questionnaires at each visit were asked which treatment they felt their pet was happiest on and they all answered DOCP with prednisolone. Initially, the plan had been to allow owners to choose which treatment they would like their dog to continue on. However, as Florinef® became unavailable during the study period and as generic fludrocortisone was very expensive, the licensed form of DOCP (Zycortal®) was released to provide an affordable treatment option for hypoadrenocorticism in dogs. As Zycortal® is a licensed treatment, fludrocortisone could not be used without using the cascade and therefore owners were no longer given this choice. Regardless of this technicality, most owners were keen to continue treatment with DOCP.

Fludrocortisone has both mineralocorticoid and glucocorticoid effects and therefore each type of steroid could not be individually titrated to each patient's specific requirements. Subjectively, patients seemed to be mineralocorticoid deficient on fludrocortisone more than glucocorticoid deficient. This is suggested by hyponatraemia and a generally increased plasma renin activity compared to the DOCP phase. It is therefore the author's view that the glucocorticoid content of fludrocortisone is too high in proportion to the mineralocorticoid content for the majority of patients, leading to side effects of glucocorticoid excess such as polyuria, polydipsia, polyphagia and hair loss. These clinical signs can be distressing to owners and may lead to dose reductions in fludrocortisone, reducing glucocorticoid supplementation to a more appropriate concentration but at the expense of causing subclinical mineralocorticoid deficiency.

Both the mineralocorticoid and glucocorticoid doses varied between patients with no relationship to weight. Therefore, DOCP and prednisolone appear to be a more appropriate treatment which allow individual mineralocorticoid and glucocorticoid dose adjustments. DOCP generally seems more potent, with several dose adjustments being required in many dogs before an optimum dose was found.

4.4 Limitations

4.4.1 Case Selection

Given the nature of the trial, primary care veterinary practices were approached to provide potential cases to enrol. This ensured patients were representative of a general population of dogs with hypoadrenocorticism however it perhaps selected dogs with owners who were extremely committed to their pets which may not have been representative of a general population of owners. In addition, patients were excluded for behavioural reasons and many dogs with hypoadrenocorticism could not be included as their owners could not comply with the intensive study protocol. As the study provided 6 months of free treatment, this also could have impacted on case selection and enrolment. Another limitation was that patients had to be stable on treatment with fludrocortisone and that no newly diagnosed patients were included. This prevented inclusion of dogs which may have benefited from treatment with DOCP as dogs can take a long time to stabilise on fludrocortisone therapy (Roberts et al., 2016). This also meant comparisons were made between patients stable on one treatment (fludrocortisone) and unstable on another (DOCP with prednisolone) at the start of each trial phase.

4.4.2 Fludrocortisone Availability

Florinef® (fludrocortisone acetate) became unavailable during the study period. Although this drug was considered to be pharmacologically identical to the generic formulation, this introduced a protocol deviation. The two drug formulations had different storage requirements which impacted on consistency within the trial. In addition, the generic drug was significantly more expensive which had a great impact on the trial budget. This meant that the washout period needed to be withdrawn following the fludrocortisone phase and prevented the two streams of the trial being identical. One patient did still have a washout period during the fludrocortisone phase of the trial and this meant that all dogs within the fludrocortisone phase did not receive identical treatment.

4.4.3 Clinical Signs Assessment

Clinical signs were considered a primary outcome measure and were critical in deciding upon glucocorticoid control in particular as no readily available blood test provided an alternative marker of control. Therefore, clinical signs were very important in our interpretation of treatment success. However, assessment of clinical signs is a very subjective tool and varies between owners both within and between households. Interpretation of clinical signs is also likely to vary between clinicians. Questionnaires were used to make assessment slightly more objective however these questionnaires were not validated for this use and instead were formulated purely for trial purposes. Validation of the questionnaire used could have improved assessment of clinical signs and would have allowed statistical analysis of clinical signs between groups. Instead, descriptive analysis was used as the assessment had been subjective.

Another limitation with assessment of clinical signs was the overlap between clinical signs of glucocorticoid and mineralocorticoid excess and deficiency. For example, polyuria and polydipsia could be suggestive of glucocorticoid or mineralocorticoid excess. Lethargy could be fitting with glucocorticoid excess or deficiency or mineralocorticoid excess or deficiency. Gastrointestinal signs could fit with mineralocorticoid excess or deficiency. Therefore, thorough histories, biochemical results and timing were used to help assist in interpretation of these signs however it was impossible to define what was causing each sign. Therefore, errors could have been made whilst considering the presence of clinical signs.

4.4.4 The Study Protocol

Several protocol deviations and amendments were required. Other than the unavailability of fludrocortisone, none of these were thought to have a direct impact on the results. One patient was included who was on a topical steroid preparation during the trial however as discussed (section 4.2.1), this was not considered to affect the results.

During the trial, although all blood samples were submitted to a reference laboratory, decisions regarding mineralocorticoid control and dosing adjustments needed to be made at the time of the visit. Therefore, bloods were also run on a point of care analyser (section 2.5.2.2). It is a limitation of this study that the electrolyte results discussed were not the exact results used to consider dose adjustments. Since there are inherent differences between analyser methodologies, it is important that the same analyser is used for dose (Fowlie et al., 2020). As the aim of this trial was to assess non-inferiority and not to review the intricacies of managing patients with hypoadrenocorticism with DOCP and prednisolone, accuracy (of the reference laboratory) rather than practicality (of the point of case analyser) was prioritised.

Although DOCP and prednisolone were non-inferior to fludrocortisone, the overall performance of this treatment method was suboptimal due to the over-treatment of many patients. Patients only remained on each phase of the trial for three months and this time was not long enough to reach the optimum dosing regimen for all patients. If there had been a longer period of time on each phase of the trial, the performance of DOCP, and likewise the under supplementation from fludrocortisone, may have become more apparent. The length of the DOCP phase was a major limitation of this trial as it did not allow for complete stabilisation of all patients.

4.5 Clinical Implications

As Zycortal® is a licensed preparation of DOCP in Europe and fludrocortisone is not licensed, this is the recommended treatment for canine hypoadrenocorticism in Europe. Fludrocortisone can be used under the cascade if required. It should however be noted that the results of this study suggest subclinical mineralocorticoid deficiency in some patients using fludrocortisone as indicated by hyponatraemic episodes and increase in plasma renin activity.

Although DOCP tended toward over supplementation of mineralocorticoids, all patients were considered to be clinically well controlled, particularly after a longer period of dose adjustments. The median dose of DOCP at the end of the DOCP phase was 1.74mg/kg every 28 days and this would be expected to be lower if patient follow up had been longer. As some patients did require a dose increase in DOCP, it is recommended that the starting dose of DOCP is not too conservative and 1.6mg/kg every 28 day seems appropriate.

When assessing the dose of DOCP, it is recommended that sodium and potassium are reviewed independently rather than using sodium: potassium ratios. This is as the results show that sodium:potassium ratios can miss patients with one electrolyte outwith the normal reference range. In addition, the upper limit of the sodium:potassium reference range of 32 is unrealistic for patients on DOCP treatment and could lead to excessive dose adjustments. Hypokalaemia or hypernatraemia at any point following DOCP dosing should lead to a dose reduction and conversely, hyperkalaemia or hyponatraemia should result in a dose increase of the drug.

No definitive drug side effects have been found during this study, although one patient was thought to develop pancreatitis at a time when the drug was at its highest systemic concentration. This condition was transient and recurrent episodes of the condition resolved with dose decreases in DOCP. Therefore, although overdosing of DOCP could potentially cause side effects such as weakness, lethargy and pancreatitis, the drug is considered to be safe and well tolerated. Suspected drug overdoses can be managed with spironolactone, an aldosterone receptor blocker.

In the future, further studies investigating the volume of DOCP dose adjustments based on in-house electrolyte measurements at day 10 and day 28 are required. In addition patients require longer follow up to first of all find their individual optimum dosing regime and second of all, to monitor the long-term stability of patients. Studies showing long term control with Zycortal® and prednisolone and whether glucocorticoid or mineralocorticoid requirements change with time are lacking. In addition, dose requirements are highly variable between patients and other methods of dosing such as mg/m^2 could be considered to assist in determining more universally appropriate starting doses for both medications. It would also be interesting to look at the use of other steroid preparations, particularly long acting steroid injections such as methylprednisolone (Depo-medrone®) which could allow for monthly injections of two forms of steroid.

In humans, adrenal autotransplantation has been attempted in the 1960's with poor success (Hardy et al., 1985). More recently, allotransplantation and stem cell biology have shown more promise for regaining functional adrenal tissue in human patients (Vouillarmet et al., 2013, Dubernard et al., 1995). Although numerous animal models have been considered (Thomas et al., 1997), these procedures are still in their infancy and maybe be worth consideration for dogs in the distant, but not near future.

4.6 Conclusion

DOCP and prednisolone have been found to be non-inferior to fludrocortisone in the management of canine hypoadrenocorticism. This is the first study to directly compare both treatment methods using a randomised cross-over trial rather than switching from one drug to another following treatment failure. In conclusion, DOCP and prednisolone offer a safe and effective treatment option for canine hypoadrenocorticism.

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