Comparing the effects of first line antiepileptic drugs on the gait of dogs with idiopathic epilepsy

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Abstract

Idiopathic epilepsy (IE) is a common chronic neurological disease of the dog. Previous studies of anti-epileptic drug (AED) treatment have indicated that acceptable AED adverse effects are as important to owners as reductions in seizure frequency. AEDs in both dogs and humans are frequently associated with the adverse effect ataxia. The aim of this study was to compare ataxia levels in dogs with IE treated chronically with phenobarbitone or imepitoin, the two currently available first-line AED treatments. The gait of six imepitoin-treated dogs, eight phenobarbitone-treated dogs and ten age-matched healthy control dogs were compared. Fifty strides from a walking gait were analysed for each dog, quantifying ataxia via the variability in six established gait parameters. Three variables differed significantly between groups: lateral distance between (i) pelvic paw placements, (ii) thoracic paw placements and (iii) stance time, which were significantly more variable in the phenobarbitone-treated dogs than imepitoin-treated or control dogs. These results indicate that dogs treated with phenobarbitone experience increased ataxia compared to controls and imepitoin-treated dogs. Conversely, there was no difference between imepitoin-treated dogs and controls. These results along with further research are needed to quantify AEDs adverse effects, to help vets and owners make more informed drug-choices.

Abbreviation	
AED	Anti-epileptic drug
BID	Twice a day
CNS	Central Nervous System
CMSM	Chiari like malformation and Syringomyelia
CV	Coefficient of Variation
IE	Idiopathic Epilepsy
MRI	Magnetic Resonance Imaging
PB	Phenobarbitone
RVC	Royal Veterinary College
QOL	Quality of Life

Introduction

Idiopathic epilepsy (IE) is a brain disease characterised by recurrent seizures that is diagnosed by exclusion of identifiable structural and metabolic causes, and can be of genetic aetiology (Berendt and others 2015; Thomas 2000). The prevalence of IE within the UK dog population is reported at 0.62% (Kearsley-Fleet and others 2013), and raw data from the Royal Veterinary Colleges VetCompass records found that seizures are the third most reported nervous system presentation in dogs (VetCompass 2016). While prevalence alone indicates the importance of this disease, IE also has potentially large welfare implications for the affected dog (Packer and Volk 2015a) and can be distressing for owners to witness and manage (Lord and Podell 1999; Wessmann and others 2014).

There are several antiepileptic drugs (AED) available for dogs with IE as monotherapies or adjunct polytherapies. Phenobarbitone (PB) and imepitoin are two licensed AEDs in the UK, Australia and most countries in Europe. Both drugs have been demonstrated to have good efficacy when used as a first-line treatment for dogs with IE (Charalambous and others 2014). PB has been shown to effectively reduce seizure frequency, but has also been associated with adverse effects such as sedation, polyphagia, polyuria, polydipsia and ataxia (Tipold and others 2015). Imepitoin has some potential benefits over PB as it has fewer reported adverse effects and does not require serum level measurements due to its high therapeutic index (Rundfeldt and Löscher 2014; Tipold and others 2015).

A study investigating owners' perception of the adequacy of seizure control, showed that quality of life (QoL) and acceptable adverse effects of AEDs were just as important to owners as a reduction in seizure frequency (Chang and others 2006). There is currently a lack of

objective data regarding AED adverse effect profiles (Charalambous and others 2014), hampering objective choice of AEDs based on both efficacy and adverse effect profile. As such, there is a strong need for more objective studies of the adverse effects of drugs including PB and imepitoin. One of the reported adverse effects of PB is ataxia (Tipold and others 2015), with one survey reporting ataxia in 30% of PB-treated dogs (Chang and others 2006).

Ataxia is a loss of control of limb coordination and position, which results in delayed and variable foot placements (Hamilton and others 2007). In human medicine, ataxia or a loss of balance associated with AEDs has been a focus of study due its association with falls and skeletal fractures (Fife and Sirven 2005; Gandelman-Marton and others 2006; Mattson and Gidal 2004). One study found that balance was impaired in epilepsy patients with AEDs compared to their non-AED treated siblings (Petty and others 2010). As yet, similar studies objectively assessing the impact of AEDs on gait have not yet been performed in dogs with IE.

The use of gait analysis to identify or quantify the severity of neurological gait abnormalities is often carried out using subjective scale systems, such as a 14-point numerical scale assessing the gait patterns observed (Olby and others 2001). This method is easily implemented in a clinical setting and has been used to assess the recovery of dogs post spinal surgery successfully (Olby and others 2004). There are concerns, however, with reproducibility of rating scales due to their subjective nature (Gordon-Evans and others 2009), observer bias (Arkell and others 2006) and the influence of observer experience on the results (Keegan and others 2010; Keegan and others 1998). Objective gait measurements are increasingly feasible due to the increased availability of high definition cameras and relevant computer software. For example, pelvic limb paw placement was successfully quantified in dogs with spinal cord injury using high definition motion cameras and markers (Hamilton and others 2007), with increased variability

in lateral paw position seen in dogs with spinal cord injury (Hamilton and others 2008). An alternative method using high speed recordings of dogs on treadmills revealed lateral instability and an increase in gait parameter variability in dogs with spinal cord injury (Jeffery and others 2011). Most recently, a combination of using a subjective scale system and high definition filming has been used to produce continuous data sets for use in future clinical trials in dogs with spinal cord injury and ataxia, as well as simplistic gait analysis methods to better quantify ataxia (Olby and others 2014; Song and others 2016; Suiter and others 2013). By formulating a method that takes into consideration gait parameters such as stride length, lateral paw placement and step cycle, it may be possible to objectively quantify the presence and degree of ataxia in dogs being treated with AEDs.

The aim of this study was to objectively evaluate the gait of dogs with IE being treated with either PB or imepitoin as monotherapy for canine IE. It is hypothesised that the PB- or imepitoin-treated dogs will show greater variability in their gait parameters compared to a healthy control dog population. This is the first known study to quantify gait parameters in dogs receiving AED therapy, and provides important data on adverse effects.

Method and Materials

Recruitment

Dogs were recruited for three separate study groups; healthy control dogs, PB-treated dogs, and imepitoin-treated dogs. Due to the variation in breed of dogs affected by IE, and the potential impact of conformation on gait, control dogs were chosen that were close in age and size to the dogs with epilepsy. Control dogs belonged to vet students and staff from the Royal Veterinary College (RVC), Hertfordshire, and IE dogs were recruited via social media and

from first opinion small animal vet practices. To be included in the study dogs were required to meet the following inclusion criteria:

(i) Between 6 months and 10 years of age

(ii) Confirmed by a veterinary surgeon to be free of orthopaedic disease based on clinical history

(iii) Dogs with IE were required to fit Tier I or Tier II confidence level of IE diagnosis (De Risio and others 2015). In brief, Tier I dogs had a history of two or more epileptic seizures with unremarkable inter-ictal physical and neurological examination with normal bloodwork and urinalysis. Tier II dogs had the same findings as Tier 1 with added diagnostics revealing normal brain MRI findings, unremarkable bile acids and unremarkable cerebrospinal fluid analysis.

(iv) Dogs with IE were required to have been treated with PB or imepitoin as a monotherapy for at least 2 weeks prior to the study.

All owners who volunteered their dogs were provided with an owner information sheet (RVC Animal Welfare and Ethics Committee reference 2015/T334) explaining the study protocol and signed a consent form to allow their dogs involvement in the study.

Gait analysis

Motion capture was performed in the structure and motion lab situated at the RVC and at owner's homes if they could not travel. Two digital stills cameras (Nikon 1-J1 model) capable of full high definition video capture were used. Both cameras were set up over 1m away from the walkway mat in order to capture both a front on, and lateral view of the dogs. The dogs were walked over-ground along an 8 m runway with black electrical tape marking up 0.5 x 0.5m squares into a 0.5 x 5 m grid made up of black tape on a flat surface. Cameras and the runway mat were fully portable so could be taken to owners' houses if required. The mat was

placed on a flat and solid surface and the camera set-up was as described above. Both cameras were set to film in full high definition, pixel 1920x1080 at 60fps, shutter speed 1/1250. Owners were then asked to walk their dog at their own comfortable walking gait on the lead. Owners were advised on how best to walk their dog along the runway, ensuring the dog remained within the mat at all times during the walk. Dogs did not require any training or markers on their bodies to be filmed. If a dog transitioned into another gait (e.g. trot or pace), stopped or became distracted during a walk, the filming was aborted and the walk repeated. 50 strides were collected in order to increase the reliability and statistical power of the results, and is similar or in excess of the number of strides used in other gait studies analysing ataxia in dogs (Hamilton and others 2007; Ishihara and others 2009; Jeffery and others 2011)

Parameters measured were chosen based on parameters measured in other neurological gait studies (Hamilton and others 2007; Jeffery and others 2011; Olby and others 2014) and are summarised in Table 1. These parameters were measured post-filming using the recorded footage, assessing each dog's gait using Quintic Video Analysis Software (Quintic Consultancy Ltd, West Midlands, UK). To analyse paw placements, freeze frames were taken at points where a paw was fully on the ground. This was performed with footage from both cameras and was performed for every foot for all strides taken. Once all the frames were collected, images were opened with software 'Image J' (ImageJ 2012). Image J allowed a scale to be set for the grid, so the image coordinates (pixels) matched real life coordinates (cm) and chosen parameters were measured using the straight line tool.

Statistical analysis

Data were collated in Microsoft Excel 2010 (Microsoft Corp, Redmond, WA, US) then analysed using GraphPad Prism (Version 5, ©1992-2010 GraphPad Software, Inc.) for univariate analysis, and IBM SPSS Statistics (Version 19, Armonk, NY: IBM Corp) for multivariate analysis. For each gait parameter the mean and standard deviation was calculated,

along with the coefficient of variation (CV = standard deviation / mean). CV is a unit-less parameter which quantifies the spread of data relating to the variability of a parameter. A higher CV value indicates more variable data. By examining only the variability of the individual's gait, and not the exact parameter measurements, differences between study subjects cannot be simply attributed to variation in size or conformation. Instead, the CV values focus on coordination consistency of the individual (Hamilton and others 2008).

CV values were checked for normality of distribution, then groups were statistically compared at the univariate level using the non-parametric Kruskal-Wallis test, with summary statistics stated as medians and 25^{th}-75^{th} percentiles. If the Kruskall-Wallis test discovered significant differences between the groups, a post-hoc Dunn's test was performed to determine which groups differed from each other. Linear mixed models were then created to further analyse the association between treatment group and gait parameters. The CV of gait parameters were included as outcome measures, with the random effect of dog ID included in all models to take into account individual variation between dogs, with the fixed effect of medication group (imepitoin, PB, or control). A p-value of less than 0.05 was considered significant in all tests.

Results

Twenty-four dogs were recruited; healthy control dogs (n=10), PB-treated dogs with IE (n=8), and imepitoin-treated dogs with IE (n=6). Details of the dogs recruited are summarised in Table 2. Ages of the dogs did not significantly differ between groups.

Gait analysis

CV of two of the six gait parameters measured were found to significantly differ between groups at the univariate level, CV of pelvic paw distance and thoracic paw distance. CV of pelvic and thoracic paw distance was found to differ significantly between the following study groups, with the PB group showing a significantly higher variability in pelvic paw distance compared to the control group and imepitoin-treated group (Figure 1 and 2, and Table 3). There were, however, no significant differences between the CV of thoracic or pelvic paw distance between the control and imepitoin groups. No significant differences were found between the groups at the univariate level for the CV of step time, stance time, stride length and ipsilateral distance (Table 3).

When individual dog variation was accounted for in a linear mixed model, including dog ID as a random effect, CV of pelvic paw distance and thoracic paw distance remained significantly different between groups, with higher variability seen in the PB group compared to controls. A further association was found between CV of stance time and treatment group, with the PB group significantly more variable compared to controls (Figure 3 and Table 4).

Discussion

This study aimed to evaluate the variability of gait in dogs with IE treated with either PB or imepitoin, in order to quantify the adverse effect ataxia. To the authors' knowledge this is the first time the gait of dogs treated for IE has been objectively quantified. The method described in this study could quantify an increase in paw placement variability, as reflected in our results. Previous studies quantifying ataxia in dogs have used treadmills to maintain speed and remove variables such as direction change or handler error (Gordon-Evans and others 2009; Hamilton and others 2007, 2008; Olby and others 2014). Using trademills is not without problems, and if time is not spent habituating animals on the treadmill this can severely alter results (Clements and others 2005) and is also time and training intensive (Buchner 1994). By using the method described in this study, walking in a familiar on-lead manner on a walkway, error due to unfamiliarity and/or anxiety is reduced and is feasible for the owner and researchers to complete. Collecting data at owners' homes also had the benefit of allowing dogs to be in their familiar environments, which is expected to reduce anxiety. Anxiety in novel environments may affect gait as dogs may exhibit behaviours such as an inability to settle, seeking exit or attempting to walk closer to their owners for security. A limitation of our study design is that the analyser for the study also recruited the dogs, and therefore was not completely blinded. However, analysis of gait was temporally apart from video capture and gait parameter measurements were objective using a consistent measurement technique.

A wide variety of breeds were recruited for this study, due to epilepsy affecting many breeds (Hülsmeyer and others 2015); however, this may be considered a limitation due to potentially significant variation in gait between breeds related to size, weight and conformation. To reduce this potential effect, we examined the CV of the individual dog's gait parameters rather than

the raw values. CV has been used to summarise the variability of foot placement during movement to quantify ataxia in people (Dubost and others 2008), dogs (Hamilton and others 2008) and horses (Olsen 2015). Hamilton and others (2008) interpreted an increase in CV values for pelvic and thoracic paws distance to be related to the inability of a dog to place their feet back into the correct lateral plane due to their ataxia. As we were able to also show a significant increase in CV values for the PB treated dogs, which we expected to be ataxic, this is supportive that our method can detect ataxia. A more recent study looking into simplified gait analysis of dogs' post-spinal cord injury (Song and others 2016) found that the CV of 'Base of Support' in pelvic and thoracic limbs (similar measurement to lateral distance used in this study) did not significantly differ between normal dogs and those with spinal cord injury. However, dogs with spinal cord injury typically have asymmetrical spinal lesions which can allow for compensation. In contrast, dogs treated with antiepileptic drugs are expected to have symmetrical deficits, due to an increase in the action of GABA (Podell 1998). This could be altering the proprioceptive pathways and their central processing, due to unspecific targeting within the CNS.

The results of this study indicate that being treated with PB affects the gait of dogs with IE. This is similar to previous findings in people (Fife and Sirven 2005; Gandelman-Marton and others 2006; Petty and others 2010) although not directly replicated. Dogs treated with PB were shown to have a significantly higher variability in their lateral paw placements (pelvic paw distance and thoracic paw distance) and stance, suggesting an inability to coordinate their paw positioning whilst walking when compared to the imepitoin treated dogs and control dogs. This finding supports our initial hypothesis, and provides objective support for the observation that PB causes ataxia in dogs with IE, as previously reported by owners and clinicians (Chang and others 2006; Dayrell-Hart and others 1991; Tipold and others 2015). In comparison, the

imepitoin-treated groups had very similar CV values to the control group. By having CV values as low as the control groups, we can assume that this is because the imepitoin treated dogs show little to no signs of ataxia. This is likely due to imepitoin's mechanism of action. Imepitoin is a partial agonist of $GABA_A$ receptors, and acts on the benzodiazepine site which only modulates the GABA effect, rather than directly activating the receptor (Tipold and others 2015). By having a much lower intrinsic activity in comparison to PB, this could explain why ataxia and other adverse effects are seen less often (Rundfeldt and Löscher 2014) and why we found no difference to our control group CV values.

CV of step time was not found to differ between treatment groups in any analysis. CV of stance time variability did not differ between treatment groups in the univariate analysis. However, when the non-independence introduced by individual dog variation was accounted for in a mixed effects model, CV of stance time was significantly more variable in PB treated dogs compared to controls. Step time and stance time have been found to be significantly reduced in mice with spinal cord injury (Beare and others 2009) as well as in ataxic dogs (Gordon-Evans and others 2009). The PB treated dogs in this study did not have reduced stance time, but more variable stance time during their walking gait, which could be explained by the large variability from stride to stride. In combination with the lateral paw placement variability, we can expect that with some wide and some narrow strides, there will be varying times that the opposing paw will be kept on the ground in order to remained balanced during the walk. The other measured parameters did not show a change in variability between the three groups. Ipsilateral paw distance variability has only previously been measured in one study, where the gait of Cavalier King Charles Spaniels with Chiari-like malformation and Syringomyelia (CMSM) was studied. That study showed a significantly higher variability in ipsilateral paws distance compared to a group of similarly-sized control dogs (Suiter and others 2013). In this

study, PB-treated dogs did not show this, which may be explained by the diffuse effect of PB on the CNS compared to a more localised pathology seen in dogs with CMSM. Stride length variability did not differ between groups, which was unexpected as an uncoordinated gait with a combination of long and short strides may be expected due to altered balance during the walk. It is likely that to see an increase in stride length variability, the dogs would have observable ataxia, as this is what has been found in previous ataxia studies (Hamilton and others 2008; Olby and others 2014; Gordon-Evans and others 2009). The PB treated dogs in this study were not reported to have ataxia by the owners, and often it is dogs who are in the first month of PB administration that show the most ataxia (Boothe and others 2012). As our dogs were chronically treated with phenobarbitone, they have had time to adjust to the drug activity and therefore only show mild ataxia which the owners might notice anymore subjectively but can be picked up by more objective assessment techniques. As well as this, previous ataxia studies (Hamilton and others 2008; Olby and others 2014; Gordon-Evans and others 2009) have looked at localised spinal pathology rather than at a diffuse reduction in CNS activity.

The PB group had a much wider range in CV values as well as higher mean CV values. This may indicate that PB treatment affects individuals to different extents, with variation in the presence and severity of ataxia. An owner survey found that PB-treated dogs had a reported ataxia incidence of 30% (Chang and others 2006), and thus not all dogs might be affected by this adverse effect or more likely owners do not recognise the adverse-effect ataxia. As aforementioned within this study none of the owners of the IE dogs declared ataxia as a problem in their dogs' lives. Two PB treated dogs were no longer achieving fast times in agility training, however the owners were confident their dogs' QoL was not affected. Further research into how PB serum levels correlate with ataxia severity would be worthwhile and it would be interesting to understand owner's subjective thresholds for reporting ataxia in their dogs.

Further research is also required to compare the prevalence and severity of ataxia seen with different AEDs available to treat dogs with IE, or of different combinations of AEDs, as many IE affected dogs require more than one AED to control their seizures (Packer and others 2015b; Platt and others 2006; Schwartz-Porsche and others 1985; Thomas 2000). Quantifying AED adverse effects is an important area of study, as IE affected dogs are treated with these medications chronically due to IEs lifelong nature, and the relative rarity of remission (Packer and others 2014). This means that chronic adverse effects may have a large and lasting impact on the QoL of the treated dog and their owner. The more that is understood about AED adverse effects, which drugs they are most commonly associated with, and which dogs are most likely to be affected by them, the more vets will be able to offer a more tailored approach to IE treatment, to best suit the individual patient.

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Table 1: Descriptors for each gait parameter measured

Stride Parameter	Descriptor
Step Time	Time between the toe of the paw coming off the ground and then the same paw being placed back on the ground. Measured in seconds.
Stance Time	Time between foot landing on the ground and then the same paw being lifted off the ground into another stride. Measured in seconds.
Stride Length	The distance from where the toe of the paw of interest leaves the ground and where the same foot subsequently lands during walk. Measured in cm.
Ipsilateral Paw Distance	The lateral distance between the thoracic limb paw placement and the ipsilateral pelvic limb paw placement during the walk. Measured in cm.
Pelvic Paw Distance	The distance between the placement of the left pelvic limb paw and the subsequent right pelvic limb paw placement. Measured in cm.
Thoracic Paw Distance	The distance between the placement of the left thoracic limb paw and the subsequent right thoracic limb paw placement. Measured in cm.

Table 2: Signalment and clinical characteristics of dogs enrolled in this study

Dog ID	Age	Breed	IE Tier and Diagnosis Year	Time treated/ months	Dose / mg/kg BID	Serum Levels / µg/ml
Control						
C1	5.5y	Northern Inuit				
C2	5y	Labrador Cross				
C3	9y	Terrier Cross				
C4	2y	Collie Cross				
C5	6y	Terrier Cross				
C6	2y	Chihuahua				
C7	6y	Jack Russell				
C8	3y	Labrador Cross				
C9	3y	Kooikerhondje				
C10	4y	Rottweiler				
Mean ± SE	**4.5±2.0**					
Imepitoin						
I1	6y	Lurcher	Tier II 2012	30	22.5	
I2	9y	Jack Russell	Tier I 2012	24	10	
I3	7y	Jack Russell	Tier 1 2014	12	10	
I4	5y	Parsons Jack Russell	Tier II 2015	1	10	
I5	6m	Dogue De Bordeaux	Tier II 2015	1	30	
I6	4y	Chinese Crested	Tier I 2015	5	20	
Mean ± SE	**5.3±1.3**			**12.2±3.5**	**17.1±2.0**	
PB						
P1	4y	Northern Inuit	Tier II 2013	24	2.5	24
P2	3y	Old English Sheepdog	Tier II 2013	22	2.5	25
P3	4y	Whippet	Tier I 2014	10	2.5	21
P4	3y	Long Haired Daschund	Tier II 2013	22	1	20
P5	4y	Parsons Jack Russell	Tier II 2014	12	2.5	22.1
P6	6y	Lurcher	Tier II 2012	20	2	15.6
P7	4y	Belgian Shepherd	Tier II 2012	32	2.5	32
P8	5y	Jack Russell	Tier II 2013	15	2	28.2
Mean ± SE	**4.1±1.0**			**19±1.6**	**2.2±0.5**	**23.5±1.04**

SE=Standard Error of the Mean, ID=Identity, IE=Idiopathic Epilepsy, BID="bis in die" (twice daily)

Table 3: Gait parameters compared between treatment groups. Coefficient of Variation (CV) of thoracic and pelvic paw distance were found to differ significantly between the following study groups, with the PB group showing a significantly higher variability in thoracic and pelvic paw distance compared to the control group and imepitoin-treated group.

Groups	Step Time	Stance Time	Stride Length	Ipsilateral Distance	Pelvic Distance	Thoracic Distance
N (total steps analysed)	485	485	458	376	530	530
Control (n=10)	0.106 (0.063-0.129)	0.101 (0.071-0.127)	0.07 (0.035-0.079)	0.08 (0.043-0.088)	**0.168 (0.119-0.175)**	**0.157 (0.126-0.164)**
Imepitoin (n=6)	0.12 (0.066-0.142)	0.1 (0.068-0.131)	0.084 (0.051-0.104)	0.077 (0.048-0.086)	**0.125 (0.097-0.181)**	**0.160 (0.081-0.172)**
PB (n=8)	0.1 (0.067-0.105)	0.149 (0.101-0.207)	0.086 (0.061-0.091)	0.076 (0.053-0.100)	**0.299 (0.197-0.327)*+**	**0.293 (0.238-0.389)*+**

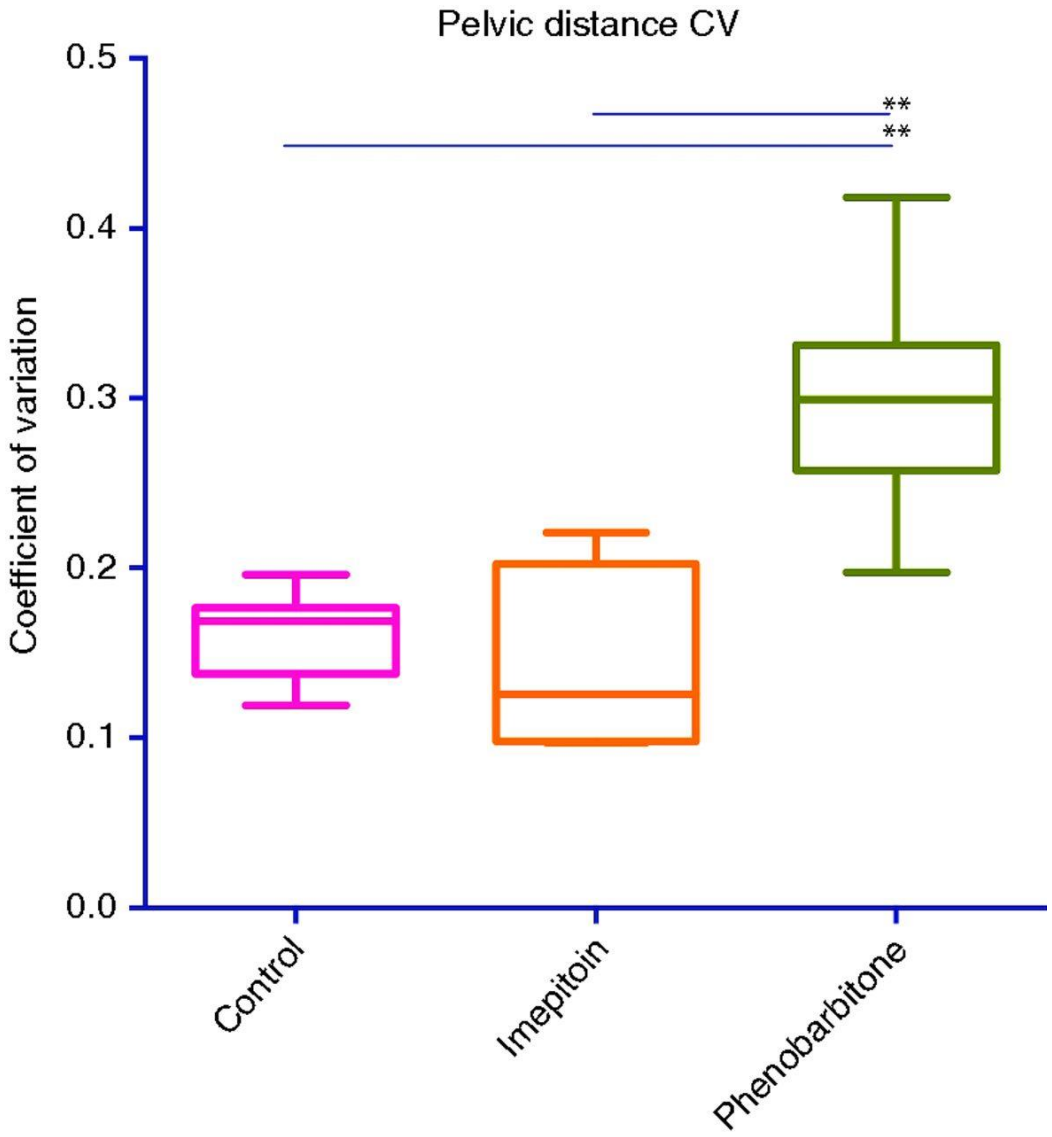
Data are presented as median (25^{th} – 75^{th} percentile) of CV values from all groups. Univariate comparison between the groups was carried out by a Dunn's Multiple Comparison Test where a p value <0.05 was deemed significant (* = significant difference to control group; + = significant difference to imepitoin group).

Table 4: Linear mixed model investigating the effect of treatment group on gait parameters, including individual dog as a random effect. Coefficient of Variation of stance time, pelvic paw distance and thoracic paw distance differed significantly between groups, with higher variability seen in the phenobarbitone (PB) group compared to controls.

Variable	**Stance Time**		**Pelvic Distance**		**Thoracic Distance**	
	CV (±SE)	P	CV (±SE)	P	CV (±SE)	P
Intercept	0.108 (± 0.033)	**0.003***	0.160 (± 0.039)	**0.001***	0.159 (± 0.055)	**0.008***
Imepitoin	0.001 (± 0.021)	0.952	-0.016 (± 0.025)	0.536	-0.011 (± 0.036)	0.770
PB	0.043 (± 0.020)	**0.046***	0.134 (± 0.024)	**<0.001***	0.168 (± 0.034)	**<0.001***
Control			*ref*			

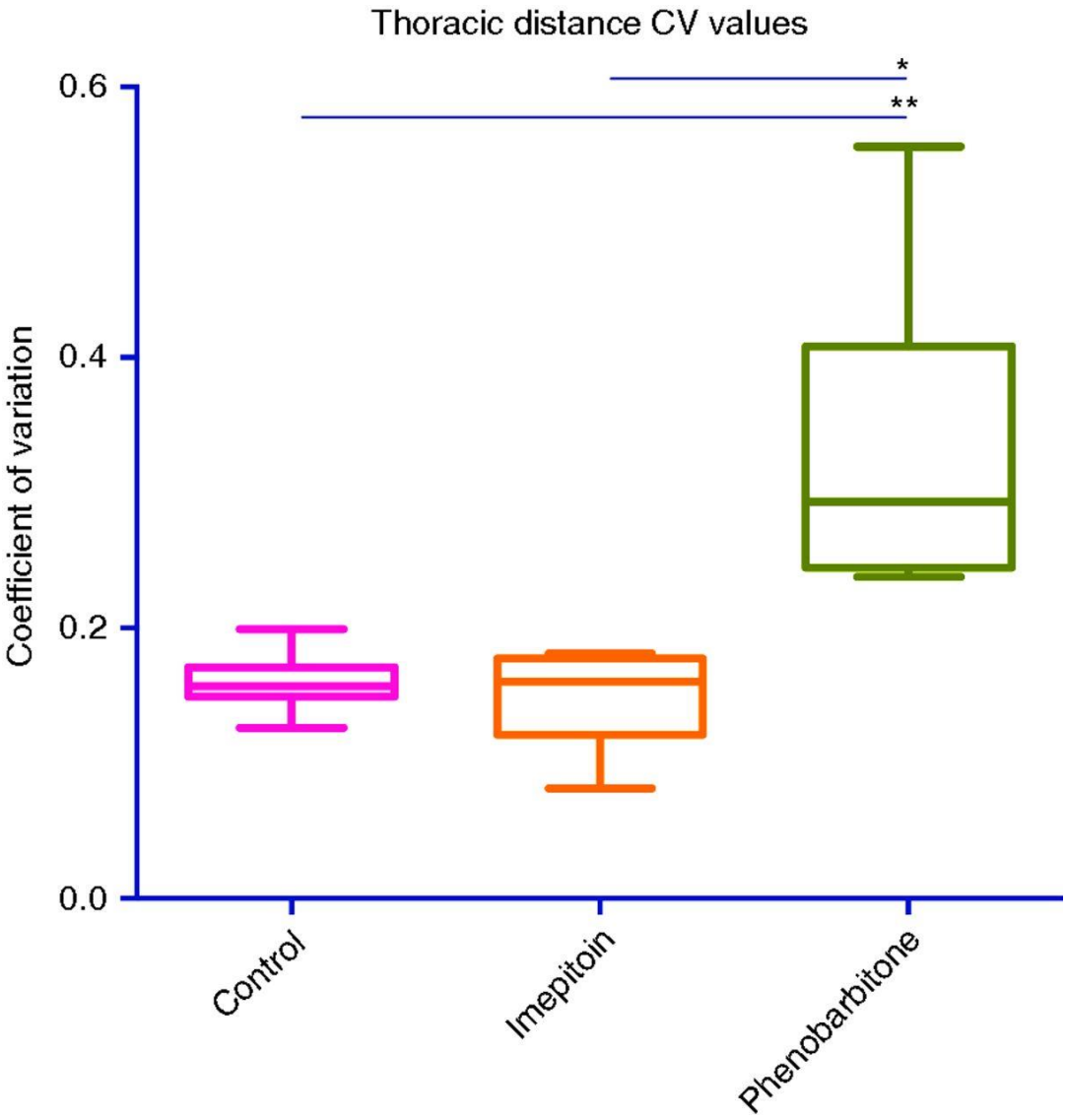
Coefficient of Variation (CV) ± Standard Error (SE) values from the treatment groups, using the control group for reference. *Linear mixed model with $p<0.05$ deemed significant.

Figure 1:



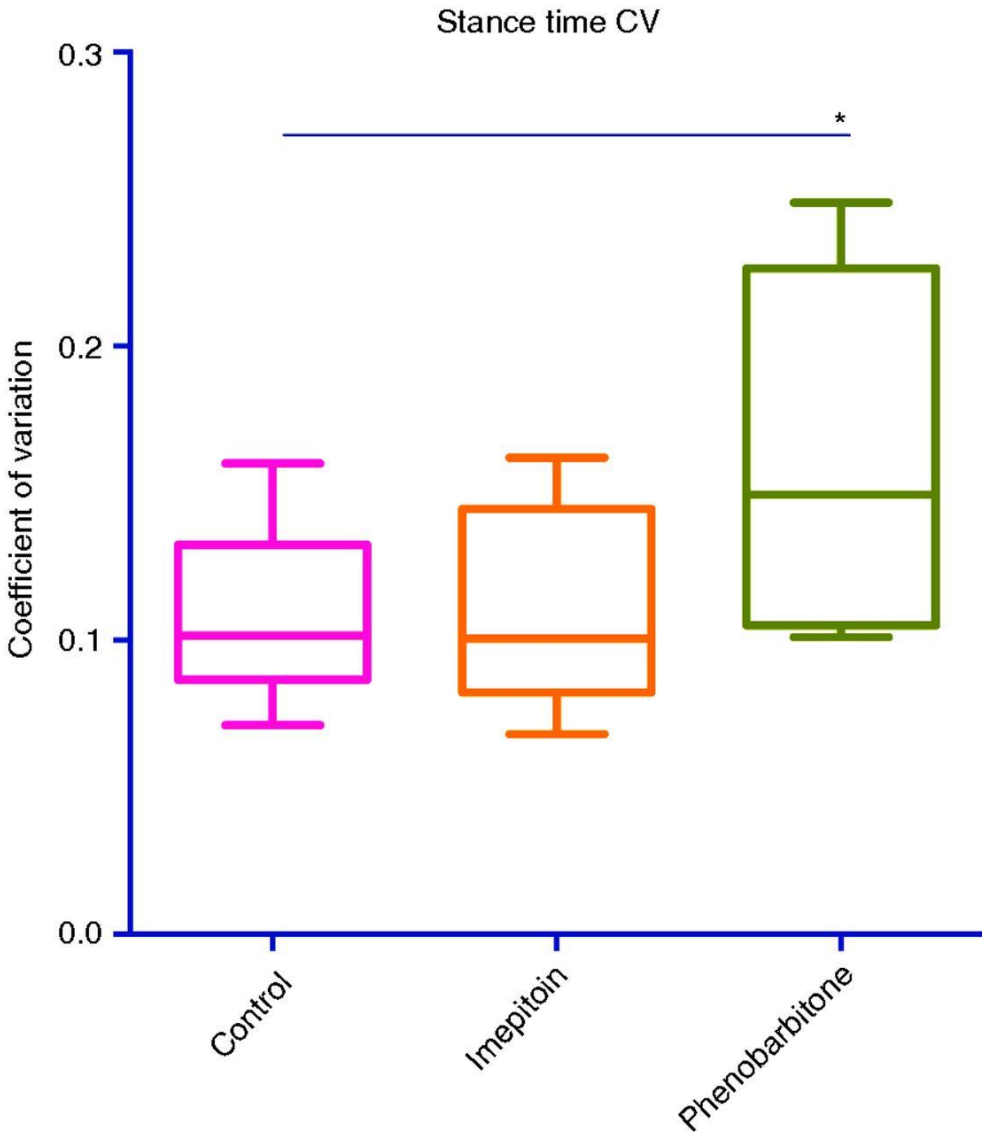
Box and whisker plots showing the comparison of pelvic paw distance Coefficient of Variation between the three study groups. Coefficient of Variation (CV) of pelvic paw distance was found to differ significantly between the following study groups, with the Phenobarbitone group showing a significantly higher variability in thoracic and pelvic paw distance compared to the control group and imepitoin-treated group. The upper and lower percentiles (95% and 5%) are shown by the coloured boxes, with median as a solid line through each box (*$p<0.05$ and **$p<0.01$).

Figure 2:



Box and whisker plots showing the comparison of thoracic paw distance coefficient of variation between the three study groups. Coefficient of Variation (CV) of thoracic paw distance was found to differ significantly between the following study groups, with the Phenobarbitone group showing a significantly higher variability in thoracic and pelvic paw distance compared to the control group and imepitoin-treated group. The upper and lower percentiles (95% and 5%) are shown by the coloured boxes, with median as a solid line through each box (*$p<0.05$ and **$p<0.01$).

Figure 3:



Box and whisker plots showing the comparison of stance time Coefficient of Variation between the three study groups. Coefficient of Variation (CV) of stance time was found to differ significantly between the following study groups, with the Phenobarbitone group showing a significantly higher variability compared to the control group. The upper and lower percentiles (95% and 5%) are shown by the coloured boxes, with median as a solid line through each box. One asterix (*) represents significance of $p<0.05$ following a linear mixed model analysis.