Validation of a New Owner-Reported Mobility Tool ("GenPup-M") Using Veterinary Clinical Examination and Quantitative Gait Analysis

'Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Masters in Philosophy'

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LIST OF ABBREVIATIONS

2D	**Two-dimensional**
3D	Three-dimensional
ACVS	American College of Veterinary Surgeons

ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BCS	Body Condition Score
BUN	Blood Urea Nitrogen
BW	Body weight
CBPI	Canine Brief Pain Inventory
COI	Canine Orthopaedic Index
CMI	Clinical Metrology Instruments
CSOM	Client-Specific Outcome Measures
CT	Computed Tomography
Cycle time	The amount of the time the foot is spent in contact with the ground and the limb bearing weight
Duty Factor	The fraction of the stride duration over which each foot remains on the ground
EC	Eithne Comerford
FP	Force plate
FPS	Frames per second
GCS	Global Coordinate System
GRF	Ground reaction force
HCPI	Helsinki Chronic Pain Index
JCS	Joint Coordinate System
JFS	Joint Function Score
Kinetic	Forces exerted from movement
Kinematic	Spatiotemporal aspects of movement
KMO	Kaiser-Mayer-Olin measure
Kg	Kilograms
LED	Light Emitting Diode
LOAD	Liverpool Osteoarthritis in Dogs
LP	Left pelvic limb
LT	Left thoracic limb
MPhil	Masters of Philosophy

MRI	Magnetic Resonance Imaging
NC	Natasha Clark
NRS	Numerical Rating Scales
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
OA	Osteoarthritis
PCA	Principal Component Analysis
Pelvic Limb	Back limbs
PID	Participant Identification Number
PVF	Peak Vertical Force
QTM	Qualisys Track Manager
QoL	Quality of Life
ROM	Range of Motion
RP	Right pelvic Limb
RT	Right thoracic Limb
SAP	Small Animal Practice
SATH	Small Animal Teaching Hospital
SDS	Simple Descriptive Scale
SEM	Standard Error of the Mean
SOAPs	Subjective Objective Assessment Plan
Stance time	The period in which the foot contacts the ground
Step Width	The period in which the food is not in contact with the ground
Stride Length	The distance between successive points of initial contact of the same foot
Swing time	The period of time when the foot first leaves the ground and ends when the same foot touches the ground again
Thoracic Limb	Front limbs
TPLO	Tibial Plateau Levelling Osteotomy
UoL	University of Liverpool
VAS	Visual Analogue Scales
PVF	Peak Vertical Force

VREC	Veterinary Research Ethics Committee
WHD	William Henry Duncan Building

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ABSTRACT

This Masters study aimed to validate a new owner-reported canine mobility questionnaire ("GenPup-M") using veterinary examination, a previously validated owner-reported questionnaire and quantitative gait analysis in dogs with/without mobility impairments.

Sixty-two family-owned dogs (31 with mobility impairments) were recruited for this study. GenPup-M responses were compared against the validated Liverpool Osteoarthritis in Dogs (LOAD) questionnaire, kinetic and kinematic gait analysis, and a validated veterinary clinical examination using Spearman's rank correlations to test criterion and construct validity. Principal Component Analysis (PCA) was used to identify if one or more question components could predict the absence/presence of mobility impairments. Cronbach's α was used to test internal consistency of GenPup-M and was found to be "good" (0.87). The PCA identified two components with Eigenvalues >1 ("stiffness/ease of movement" and "willingness to be active/exercise"). There was a strong, positive correlation between GenPup-M and LOAD responses (r^2 = 0.69, $p<0.001$), and GenPup-M and clinical examination scores (r^2 = 0.74, $p<0.001$). There was a moderate, positive correlation between GenPup-M responses and peak vertical force (PVF) (r^2 = 0.43, $p<0.001$). GenPup-M positively correlated with gold-standard objective forms of gait assessment (quantitative 3D gait analysis, validated veterinary clinical examination and a validated canine mobility questionnaire (LOAD)), adding further evidence towards validation of GenPup-M for use in all canine mobility impairments. The University of Liverpool and Dogs Trust are also working in conjunction to try and develop GenPup-M into a smartphone App to facilitate rapid owner-reported mobility assessment. There is also scope to further the use of GenPup-M to collect information relating to risk factors (e.g. walk duration/frequency, body condition score (BCS)) associated with early onset canine mobility impairments.

Quantitative canine gait analysis showed that there were statistically significant differences between peak vertical forces (PVF) of mobility impaired and non-mobility impaired dogs ($p<0.05$). Analyses of PVF showed that dogs with mobility impairments more evenly distributed their weight across all four limbs, compared to non-mobility impaired dogs. There were also differences in spatiotemporal parameters; stance time and swing phase were generally lower for the mobility impaired cohort, compared to the non-mobility impaired cohort, suggested that mobility impaired dogs were walking slower than non-mobility impaired dogs.

CHAPTER ONE | INTRODUCTION

1.1 Mobility Impairments in Dogs

In human medicine, mobility impairments are defined as a disability which can affect movements ranging from gross motor skills, such as walking, to fine motor movement such as manipulation of specific objects using hands and fingers. Consequently, this can impact a person from living a normal life and can put them at a disadvantage compared to other members of society (Sammer et al., 2012). In veterinary medicine, when determining if the animal has a mobility impairment, it is important to assess the animal's ability to engage in daily activities and interactions, and to move and exercise freely. If an animal cannot play, exercise or express normal behaviour without having a certain element of pain or reduced movement, then it can be considered that its mobility is impaired (Gruen et al., 2015). Many studies have also investigated the impact of reduced mobility on the animal's quality of life (Mullan, 2015; Fulmer, Laven and Hill, 2022; Spofford et al., 2013) and found that owners perceive improvements in mobility with an increase quality of life.

There are many factors which can lead to a dog developing a mobility impairment. For example, dogs with chronic osteoarthritis may be considered to have mobility impairments due to abnormal stresses causing chronic inflammation (Rychel, 2010). Similarly, dogs with neurological diseases can be considered mobility impaired, especially if they have conditions which cause ataxia or paraplegia, preventing them from completing activities which require normal mobility. However, in recent years, there has also been research examining the effects of extreme breeding strategies to obtain breed standards. For example, Humphries et al., (2020) found that German Shepherd Dogs (GSDs) were found to have reduced mobility at the L7-S1 region than in dogs of other breeds, and reduced hip flexion angles. Although, further studies are required to compare caudal spinal motion to other breeds.

Orthopaedic and neurological conditions are not the only causes of mobility impairments in dogs, since other breed characteristics can reduce mobility indirectly. Two studies (Liu et al., 2015; Riggs et al., 2019) investigated the impact of brachycephalic obstructive airway syndrome (BOAS) in brachycephalic breeds. Both studies found that the conformational changes associated with extensive brachycephalic breeding (elongated soft palate, stenotic nares, everted laryngeal saccules and laryngeal collapse) were all factors for reduced exercise tolerance and thus, could be considered to affect mobility.

The prevalence, aetiology, diagnosis and treatment of specific diseases which can cause canine mobility impairments will be discussed extensively throughout Chapter One, leading

to an overview of why the work proposed for this MPhil project is important to maintain (and promote) the health and welfare of dogs suffering from mobility impairments.

1.1.1 *Prevalence of Mobility Impairments in Dogs*

Musculoskeletal-related disorders are the third most prevalent disease in first-opinion veterinary practices, making up 11.8% of all cases seen (O'Neill et al., 2014). The appendicular skeleton (17.5%) is the third most common site for musculoskeletal-related problems, with the head and neck (32.8%) being most prevalent (Anderson el al., 2018). The incidence rate of canine lameness is thought to be 56% in small animal medicine (Mohsina et al., 2014). In the majority of cases, osteoarthritis (OA) has been found to be the most prevalent musculoskeletal condition in geriatric dogs (Marcellin-Little et al., 2014). The above epidemiological data highlights the requirement for early preventative actions and therapeutic interventions.

It is estimated there are approximately 12.5 million dogs in the UK (2021 Pet Food Manufacturers' Association), therefore, the prevalence of conditions leading to mobility impairments poses a risk to canine health and welfare (Summers et al., 2019). However, the prevalence in dogs is conflicted in the literature (Anderson et al., 2018); estimated percentages range anywhere from 6.6% in primary-care small animal practices, to 20% in referral settings (Johnston, 1997; O'Neill et al., 2014). A recent study calculated annual period prevalence at 2.5%, equating to 200,000 dogs affected with mobility problems, such as OA annually (Anderson et al., 2018). A study conducted in 1997, based on data collected from 200 veterinary surgeons in North America suggested that 20% of all dogs over one year old suffered from an orthopaedic condition which caused mobility impairments, this subsequently increased to 80% by the age of eight years old (Johnston, 1997). However, these statistics show statistical biased as they are determined from radiographs in referral settings, rather than clinical examination alone (Anderson et al., 2018). In recent years, prevalence estimates suggest the above figures are highly underestimated due to the methodology used in reporting cases such as OA (O'Neill et al., 2014; Anderson et al., 2018). A study by O'Neill et al (2014) estimated that up to 50% of dogs are diagnosed with some degree of reduced

mobility between the ages of eight to thirteen. A longitudinal study on 48 Labrador retrievers in 2006, showed 15% of Labrador retrievers had radiographic evidence of hip OA by two years old, further rising to 26% by five years old (Smith et al., 2006). Furthermore, 67% of Labrador retrievers were reported to suffer from OA by the end of their life.

1.1.2 *Aetiology*

As discussed in Section 1.1, the cause of canine mobility impairments is not always direct, however, many orthopaedic diseases which cause reduced mobility often have complex and specific pathways (Anderson et al., 2020). In many cases, conditions such as OA or degenerative joint disease (DJD) cause progressive degeneration of joints, which leads to remodelling, impaired mechanical function, and chronic pain (Rychel, 2010), resulting in the dog potentially being unable to move naturally or without pain.

Factors increasing the risk of dogs developing mobility impairments can be exacerbated by both genetics and lifestyle (Sanderson, 2012). Therefore, it is important to understand the predisposing risks, as disease processes and pre-existing health conditions can often influence the pathogenesis of orthopaedic conditions (Anderson et al., 2018). For example, there are two types of OA in dogs: primary and secondary (Grondalen and Lingass, 1991). Primary OA is described as being predominantly idiopathic, but is also commonly associated with certain risk factors, including age and obesity (Martel-Pelletier et al., 2016.). However, both obesity and age can increase the risk of mobility impairments developing independently. Secondary OA is believed to be the most common type occurring in dogs as the pathogenesis is considered to have a genetic component that is exacerbated by underlying disease processes or injuries that can aid the development of OA (Sanderson et al., 2012; Anderson et al., 2020). For example, hip and elbow dysplasia are both considered abnormalities within the joint which can exacerbate lameness by causing damage to the articular cartilage, and single and focal chondral defects. However, this can progress to more advanced degenerative diseases and end-stage disease (Minas, 2011), resulting in visible mobility impairments. Genetic influences include specific breeds which have a predisposition to developing conditions such as OA, hip/elbow dysplasia and cruciate ligament rupture and include Labrador retriever,

German Shepherd, Bernese Mountain Dog and Golden retriever (among others) (O'Neill et al., 2014). Increased bodyweight is also thought to have adverse effects on the development of orthopaedic disease with larger breeds more predisposed (O'Neill et al., 2014). Similarly, obesity may itself have a direct impact on causing mobility impairments, since obese dogs exercise less frequently and for shorter periods (German et al., 2017).

1.1.3 *Diagnosis*

Changes in a dog's gait can often be subtle, thus, identification of very early onset gait abnormalities by owners or veterinary surgeons can be challenging (Gruen et al., 2015) as most conditions causing mobility impairments can be falsely represented as solely affecting older dogs (Mele, 2007). Furthermore, it is thought that breeds predisposed to orthopaedic disease (as described in Section 1.1.2) are less likely to have mobility impairments recognised by owners due to the assumption this is normal within that specific breed (Packer et al., 2012). Consequently, these breeds have progressive joint dysfunction before being presented to veterinary surgeons (Packer et al., 2012).

1.1.3.1 *Imaging*

Radiography has always been the mainstay of diagnostic imaging for clinicians to investigate orthopaedic concerns in small animal practice (Fujita et al., 2005). The radiographic features of mobility impairments are generally non-specific, however, secondary to another inciting cause (Hayashi et al., 2018). Effusion, osteophytosis, intra-articular mineralisation, sclerosis and cyst formation of subchondral bone, narrowing of joint spaces and enthesophytosis are all considered to be meaningful in the diagnosis of conditions such as OA (Morgan, 1968; Bennet, 1988). In some cases, flexed, extended and orthogonal views are required to observe these abnormalities (Fujita et al., 2005).

Aggregate scores of orthopaedic pathologies are given based on the appearance and severity of the joint, with a particular focus on osteophyte formation (Wessely et al., 2017), although

the pathological process of osteophyte formation in veterinary species is not fully understood (Nganvongpanit et al., 2014). The grading systems are usually an arbitrary assessment, thus, there is a compromise between reliability (due to subjectivity) and sensitivity (Allan and Davies, 2018). Veterinary studies have investigated the use of these scoring systems in dogs, however, they remain on a relatively small scale (Widmer et al., 1994; Dendrick et al., 1993; Innes et al., 2004; Wessely et al., 2017). Most commonly, the loss of the infrapatellar fat pad, resulting in joint effusion, and osteophyte formation were the earliest radiographic changes relating to stifle OA (Widmer et al., 1994; Dendrick et al., 1993; Heffron et al., 1979; Marshall, 1969; Gilberton, 1975; Innes et al., 2004; Allan and Davies, 2018).

However, radiographic changes may not always equate with clinical evident orthopaedic pain (Widmer et al., 1994; Dendrick et al., 1993) as cartilage degradation is not visible, nor are some mild changes (Allan and Davies, 2018). Therefore, to identify clinically significant lesions, a more sensitive modality may be required, for example, computerised tomography (CT) or magnetic resonance imaging (MRI) (Hayashi et al., 2018); compositional MRI imaging allows assessment of both structural and compositional cartilage changes, when perhaps, the orthopaedic disease is asymptomatic, whereas, CT identifies later stages of the disease when microscopic bone remodelling or osteophytosis occurs (Jones et al., 2022). Regardless, both MRI and CT allow the identification of earlier stages of orthopaedic disease progression when compared to radiography and clinical examination. However, imaging is still not considered as sensitive as biomarkers of orthopaedic disease Lotz et al., 2013.

1.1.3.2 *Biomarkers*

Research into biomarkers of osteoarthritis is currently being investigated in both human and veterinary medicine (de Bakker et al., 2021). Biomarkers aim to quantify joint remodelling and disease progression to better understand collagen metabolism in cartilage and bone, with other biomarkers being related to non-collagenous proteins, inflammation and/or fibrosis.

Biomarkers in OA specifically may help to categorise the burden of disease, aid investigation, prognosis and efficacy of surgical or medical interventions (Lotz et al., 2013). In 2021 de Bakker et al. conducted a pilot study to assess synovial fluid biomarkers of disease and non-disease canine joints and compared the findings to clear radiographic changes of OA. The study found no correlations between the biomarkers and the radiographic scoring system of OA; therefore, future research is required to determine the benefit of biomarkers in the early detection of canine orthopaedic disease (de Bakker et al., 2021).

1.1.4 *Treatment*

Depending on the causative factor, mobility impairments can be treated either surgically or conservatively, and a quality of life assessment should be conducted prior to any large surgery (Roberts et al., 2021). It is estimated that 85% of mobility impairments due to cranial cruciate ligament (CCL) rupture are treated with either a surgical or medical modality, with 77.9% receiving analgesic medication (most commonly non-steroidal anti-inflammatories (NSAIDs). Surgical management occurred in 4.8% of cases, with 74.3% remaining on medical management. Thus, medical treatment is suggested to be the mainstay treatment of orthopaedic conditions causing mobility impairments at present (Anderson et al., 2018). Dog owners also need to understand that many mobility impairments are lifelong ailments that cannot be cured, just managed (Rychel, 2010). However, it must also be highlighted to owners that pain associated with orthopaedic disease does not have to be a part of their dog's normal ageing process and that conservative and/or surgical intervention can be applied to ensure their dog is kept comfortable and to maintain a certain element of mobility (Roberts et al., 2021). In the early development of orthopaedic disease or prophylactically in specific breeds (i.e. Labrador retrievers), some veterinary surgeons may advise the owner to give their dog(s) nutraceuticals, since they have little-to-no side effects (Elrod and Hofmeister, 2019).

1.1.4.1 *Nutraceuticals*

Until now, therapeutic management of OA in dogs has mainly been dominated by nonsteroidal anti-inflammatory drugs (NSAIDs). However, recent studies in human medicine have found that avocado/soybean unsaponifiable substances can have symptomatic effects in knee OA, and could offer an alternative approach in the prevention of OA progression by decreasing matrix metalloproteinase production and increasing TGF-β1 (Boumediene et al., 1999; Hamoud, 2020). However, their use in dogs remains unexplored.

Omega-3 fatty acids are also commonly used in human medicine to help reduce chronic inflammation within joints (Gammone et al., 2018; Loef et al., 2018), since it is considered to have pharmacological action of incorporating into cells that make up the synovial membranes and other joint-related structures (Loef et al., 2018). Thus, this reduces the magnitude of the inflammatory cascade at the site (Loef et al., 2018). A 2008 study, investigated the benefit of increased dietary fish oils, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in dogs with CCL injury pre-and post-surgery. The results of the study found that matrix metalloproteinase activity did not alter in the surgically-operated stifle, however, it had significantly decreased in the non-surgical stifle, suggesting that the implementation of dietary fish oil may have beneficial effects on the synovial fluid equilibrium in the uninjured stifles of dogs with unilateral CCL injury, and thus, may have some advantage prophylactically (Hansen et al., 2008). Similarly, a double-blinded study researched the effects of Omega-3 fatty acids on weight-bearing dogs with OA. The study showed that mean peak vertical force (PVF) significantly improved in dogs who were supplemented with Omega-3 (Roush et al., 2010), however, this was only a relatively short study.

Glucosamine and Chondroitin are other common nutraceuticals used as supplements in the treatment of orthopaedic disease, for example, *in vitro* they aim to increase the production of proteoglycans by chondrocytes and reduce metalloproteinases synthesis. Verbruggen et al., 2002, found that glucosamine slows down the joint space narrowing in human knee OA, suggesting that it may slow down OA progression. The same study also found that chondroitin decreased the number of patients who developed overt erosive OA, suggesting that it may have a protective effect on joints.

The above research has demonstrated that nutraceuticals may have the potential for inhibiting structural changes of OA and other related orthopaedic conditions, though they often have a delayed onset of action (6-8 weeks). Of course, further research is required into

their significance, however, since NSAIDs have shown to have potentially serious adverse effects after prolonged administration, nutraceuticals may provide a safer and natural alternative in preventing OA progression in dogs.

1.1.4.2 *Pharmacologic methods of analgesia*

In many cases of canine mobility impairments, NSAIDs are often used as a first-line treatment of pain because of the integrated role of the COX pathway in the generation of inflammation and the biochemical recognition of pain (Ong et al., 2007). Like many other medications, NSAIDs have side effects associated with their use, such as gastrointestinal ulceration and perforation (Anderson et al., 2018). They are also contraindicated for use in patients with compromised renal functions due to predominantly being metabolised by the kidneys (Dodd, 2010). Therefore, routine haematology and biochemistry monitoring is advised every 6-12 months to ensure the dog will still tolerate the NSAID medication (Dodd, 2010).

In circumstances where animals are affected by hepatic, renal or gastrointestinal disease or those who do not tolerate the use of continuous NSAID administration, there are numerous other pharmacological pain relief alternatives (Lomas and Grauer, 2015). Additionally, almost all NSAIDs are not compatible for administration alongside corticosteroid treatment. Therefore, these should not be used concurrently and may have to supplement with other forms of analgesia (Narita et al., 2007; Johnston and Budsberg, 1997). Other analgesic options include gabapentin, amantadine and tramadol (Lascelles et al., 2008; Moore, 2016). However, gabapentin is commonly used to treat chronic pain which has a neurological component, for example, neuralgia, which cannot be controlled by NASIDs or opioids (Johnston and Budsberg, 1997).

In 2020, a novel, quick-acting, monthly injectable medication was licensed for use in dogs in the EU. Bedinvetmab (Librela®) acts on monoclonal antibodies designed to recognise and attach to a protein called nerve growth factor (NGF) and is formulated to alleviate osteoarthritic pain in dogs (Palmer, 2020). Bedinvetmab binds to nerve growth factor (NGF) and functions like naturally occurring antibodies, with minimal involvement of the liver and kidneys for its metabolism, due to it acting similarly to endogenous proteins (Palmer, 2020).

The use of Bedinvetmab has been found to significantly reduce the risk of gastrointestinal ulceration (Schmelz et al., 2019). However, advances in technology have meant that the costs of developing monoclonal antibody therapies have decreased dramatically, thus, allowing the management of conditions such as OA more under the supervision of a veterinary surgeon (von Loga et al., 2019). This may enable a holistic approach to increase the quality and safety of managing a debilitating disease (de Bakker et al., 2021).

1.1.4.3 *Multimodal approach to managing osteoarthritis*

Pharmacologic analgesia is not without risk and should only be started at the discretion of the veterinary surgeon (Anderson et al., 2018). Therefore, nonpharmacological pain relief should also be considered in milder cases of osteoarthritis where detection has been early (Rychel, 2010). **Low-level laser therapy and acupuncture** are common methods of reducing mild pain, and can both be performed in a first-opinion small animal practice environment (Anderson et al., 2018) and increase mobility and exercise tolerance. Laser therapy is also minimally invasive, and therefore, generally well-tolerated in companion animals (Hegedűs et al., 2009; Dodd, 2010). Furthermore, acupuncture provides an excellent modality to achieve pain management, again, the use of acupuncture needles is generally well tolerated by animals (Roynard et al., 2018).

Weight loss is an important factor in the management of osteoarthritis, as additional weight places abnormal stress on joints that are already chronically inflamed and painful (Marshall et al., 2010). Furthermore, research has shown that adipose tissue is pro-inflammatory and thus, can produce metabolically active cytokines (Yoon, 2014; Maki et al., 2020). Animals who are obese are usually less active, and the state of chronic inflammation can adversely affect joint health (Vuolteenaho et al., 2009). Dog owners should also be made aware that weight loss can help reduce clinical signs of orthopaedic disease and subsequent mobility impairments (Marshall et al., 2010).

Physical rehabilitation, including hydrotherapy and physiotherapy, can help maintain muscle mass and reduce pain associated with orthopaedic disease (Navarini et al., 2017). Stress on affected joints is significantly reduced in water, enabling greater range of motion (ROM),

improved resistance to build strength, and improved gait by extending stride length and decreasing pain (Millis and Ciuperca., 2015). Despite this, in some instances, it is important patients are receiving adequate pain relief medication to ensure their pain is not being exacerbated by the rehabilitation programme (Budsberg and Bartges., 2006). Owners can be actively involved in the physical rehabilitation process, and can continue the strengthening exercises at home (Rychel, 2010).

1.2 The Use of Gait Analysis in Veterinary Medicine

A full canine gait cycle comprises of two main phases, stance time and swing phase. Stance time represents around 60% of the whole cycle time, it is defined as the amount of time the foot is spent in contact with the ground. Swing phase begins when the foot first leaves the ground and ends when the same foot touches the ground again. The swing phase makes up the other 40% of the gait cycle. Both stance and swing phases are required to make one complete gait cycle (Nunamaker and Blauner, 1985). Stance time and swing phase can further be divided into foot-strike, break, propulsion and toe-off (DeCamp, 1997) (Figure 1.1). The gait cycle begins when the foot first contacts the ground. Breaking happens quickly after contact as is deemed the start of the stance phase. Propulsion is required to make forward momentum. Toe-off represents the point at which the foot breaks contact with the ground and the end of the stance phase. In canine gait, the thoracic limb provides higher braking forces than propulsion, while conversely, the pelvic limb delivers a large quantity of propulsion, but little breaking force (DeCamp, 1997).

Walking and trotting gait events are often considered symmetrical events due to the movements on one side being replicated on the contralateral limbs, however, some dogs may walk with ipsilateral gaits (DeCamp, 1997). Galloping is categorised as asymmetrical due to the gait patterns not being repeated on the opposite side. Subsequently, gallop is not used in veterinary studies to assess canine gait (Nunamaker and Blauner, 1985) as they are classed as non-sequential, non-repetitive movements (Imhof et al., 2007).

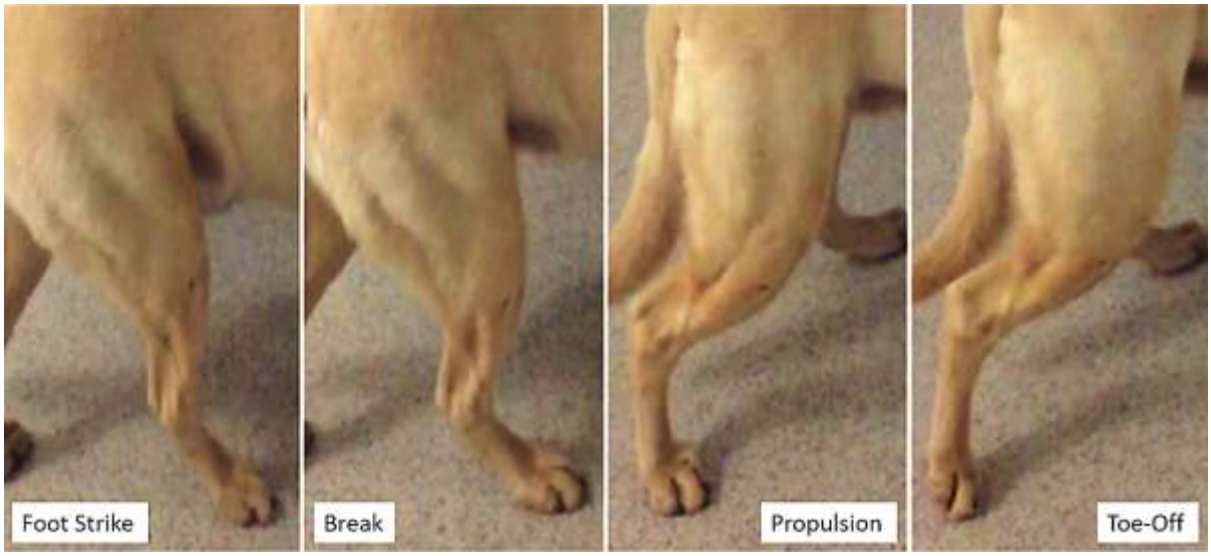

Figure 1. 1. An Illustration to show the four stages in a single canine gait cycle.

When assessing gait and mobility, movements and postures associated with daily activities should be assessed (Millis, & Weigel, 2014). Consideration should also be given to dynamic transitions associated when transferring the body to and from certain positions, for example, sitting to standing (Hesbach, 2007). When an animal is in a normal stance position, the centre of gravity is located in the mid-chest behind the scapula, this results in each thoracic limb bearing 30% of the total body weight, and each pelvic limb bearing 20%, however, there are specific breed-to-breed variations (Gillette & Angle, 2014). In clinical practice, performing a clinical examination is paramount to determining a differential diagnosis list and highlighting abnormalities (Marcellin-Little, 2020). **Clinical history** can often be an important diagnostic aid in determining orthopaedic conditions which cause mobility impairments (Belshaw et al., 2020), therefore, centring questions on the variation of stiffness throughout the day, difficulty when performing certain activities or intolerance to exercise may help owners to give detailed descriptions of their dog's mobility impairments (Cachon et al., 2018; Forster et al., 2012). If the dog is not clinically diagnosed with an orthopaedic condition, subjective-objective-assessment-plans (SOAP) can be used (Chamberlain and Yates, 2000). The use of SOAPs combined with regular evaluation and assessment of 'healthy' dogs at regular time points, has shown to be beneficial in promoting early intervention and evolution of diseases (Spofford et al., 2011).

Visual gait analysis is one of the most common forms of assessing mobility within a veterinary setting (Anderson et al., 2015); the analysis should take place in a large area with non-slip surfaces, the animal should be observed a rest, walking and trotting in a straight line, and the gait should be observed from the front, back, both sides (Marcellin-Little, 2020) and by making the animal walk in tight circles (Millis and Mankin., 2014). Additionally, any routine activities such as climbing stairs, walking up and down ramps, sitting and turning should also be assessed, to allow identification of more orthopaedic pain that may be hidden otherwise (Marcellin-Little, 2020). Furthermore, head movements are also important to assess when analysing gait, a characteristic head nod will occur in an upwards direction when thoracic limb lameness occurs, and a downwards head nod will be present in pelvic limb lameness (Mills et al., 2020). The purpose of the head nod in upwards and downwards positions respectively occurs due to the animal attempting re-locate its centre of gravity and subsequently, reduce the weight-bearing on the affected limb (Mills et al., 2020).

Following a visual assessment of gait, a hands-on **veterinary examination** should be undertaken. Harris et al (2018) used a validated clinical examination to assess joint function score (JFS) adapted by Impellizeri et al (2000) where '0' represented the normal range of motion (ROM) and '4' signified three to four joints abnormalities and a pain response was elicited upon touching the joint. Mobility/lameness scores in this validated clinical examination (0-10) were adapted from previous studies (Vasseur, 1993) to determine the dog's ROM. Visual gait analysis can be conducted in the consultation room during history taking to reveal subtle lameness, including assessment of stand-up/lie-down phases (Hazewinkel, 2003). Gait analysis is commonly performed before a physical examination as lameness may be exacerbated following manipulation of limbs (Vasseur, 1993). Importantly, dogs can adjust their gait to compensate for abnormalities associated with developing mobility impairments (Moreau et al., 2014). These coping mechanisms may cause complex pathologies if sustained for a prolonged period, emanating from the affected region to other areas of the body (Meeson et al., 2019).

Since many orthopaedic conditions which cause mobility impairments are secondary to an inciting cause, it is important that this cause is initially identified (Moreau et al., 2014). Furthermore, evidence of joint effusion, muscle atrophy, reduced ROM and joint thickening are all clinical and physical signs associated with canine mobility impairments (Quinn et al.,

2007). Additionally, superficial and deep muscle palpation should be performed to assess tone, temperature and painful areas, this should be performed when the dog is relaxed to reduce tension (Boyd, 2014). Numerical rating scales (NRS) and visual analogue scales (VAS) can be used during mobility assessments to give subjective and semi-quantified results (Hielm-Björkman et al., 2003; Quinn et al., 2007; Sharkey, 2013). Gait can be scored using a 5-grade score system of walk and trot separately, where 0 = normal and 5 = continuous non-weight-bearing lameness (Millis and Mankin, 2014). However, it is important to state that the gold standard approach for quantifying mobility problems is via force plate analysis (Waxman et al., 2005), as one study (Evans, Horstman and Conzemius (2005) evaluated visual observations of gait compared to force plate analysis in 148 Labrador retrievers (n- 131 dogs six months post-surgery for unilateral cranial cruciate ligament injury). The results showed that the observers only identified 11% of the 131 dogs who underwent surgery as having abnormal gait, whereas the force plate data revealed that 75% of the 131 dogs had peak vertical force less than that of sound Labrador retrievers.

1.2.1 *Introduction to Canine Gait Analysis in Veterinary Medicine*

In both human and veterinary practices, there are concerns that the grading of lameness is a subjective practice and that clinicians develop their own parameters and scoring systems (Waxman et al., 2006); this can be further complicated by the speed at which multiple gait events occur during the locomotion phase (Kano et al., 2016). A study conducted in 2006, found that (with the exception of severe lameness) visual gait assessment was largely inaccurate (Waxman et al., 2006). Therefore, a more quantitative approach (e.g. force plates) is sometimes used to support the visual examination (Hercock et al., 2009; Waxman, 2008).

Objective gait analysis can either be via kinetic or kinematic evaluation (Kano et al., 2016). Objective analysis using either method, predominately focuses on the foot, as this is the terminal section of the thoracic and pelvic limb (Besancon et al., 2004). In any case, it is important to state that the foot is a complex structure which provides support, balance and propulsion in the stance and gait phases respectively (McLaughlin, 2001). Additionally, load patterns are constantly changed throughout locomotion from one paw to another (Besancon et al., 2004). **Kinetic gait analysis** involves the use of force plates to produce computational

measurements of ground reaction forces (GFR) (Bockstahler et al., 2009), whereby, the ground reaction forces are used to quantify the force applied to each foot during an individual strike to the ground (McLaughlin, 2001). Although, when using one force plate, only ipsilateral food strikes can be assessed during one trial (Richardson, Boston, Kapatkin, 2004). Therefore, canine kinetic gait analysis is often considered time-consuming and laborious (Richardson, Boston, Kapatkin, 2004). Kistler developed one of the first instruments to be used in veterinary gait analysis, a treadmill with integrated force plates (Richardson, Boston, Kapatkin, 2004). This instrument allows rapid production of ground reaction forces for all limbs, thus, decreasing the time spent on data collection (Waltraud, Matis, 1997).

In contrast, the collection of **kinematic gait analysis data** uses either two-dimensional or three-dimensional (3D) motion cameras to assess complex body segment motions involved in habitual gait (Sandberg et al., 2020). 3D kinematic gait analysis requires specialist equipment, predominantly markers (coloured, light-emitting diode (LED) or retro-reflective) that are placed onto the dog's skin at specific anatomical landmarks (Kim et al., 2017). Common anatomical locations for marker placement include cervical vertebrae 7, thoracic vertebrae 13, lumbar vertebrae 7, the dorsal spine of the scapula, acromion, medial and lateral humeral epicondyle, ulnar styloid, the wing of the ilium, greater trochanter, medial and lateral femoral epicondyle, femorotibial joint, lateral malleolus of the distal tibia (Sandberg et al., 2020). Markers are also frequently placed on the 5th metacarpal bone of the toe (Kim et al., 2017). The markers are detected by specialised motion-capture cameras when a dog is walked into the 3D testing space (Sandberg et al., 2020). Specialist cameras are linked to video processors and computers to enable the linear transformation to determine the relative positions of each target in 3D space (McLaughlin, 2001). There are huge structural variations between, and within dog breeds, thus making comparisons difficult within a varied cohort of dogs (Keebaugh et al., 2015). Some veterinary studies have used kinematic gait data to assess normal canine gait at different speeds, and to evaluate mobility impaired dogs who have suffered from cranial cruciate ligament ruptures (Gordon-Evans, 2012; Gillette, 2008).

1.2.2 *Kinetic Gait Analysis*

Kinetic gait analysis allows the quantification of varied forces that exist internally and externally in a biological system (McLaughlin, 2001). Kinetic variables commonly known as ground reaction forces include peak vertical forces, peak horizontal forces, vertical impulses, loading rates, distribution of forces over the paw and specific characteristics relating to temporal gait events. Peak vertical force (PVF) is the largest force obtained while a dog is in the stance phase, data is represented as a single-data point occurring during a force-time curve, and vertical impulse is calculated by using the area under the vertical force curve using time. Peak vertical force and vertical impulse are most commonly used in kinetic gait analysis to detect lameness, generally, dogs who have mobility impairments resulting in lameness have lower peak vertical forces and vertical impulses in the affected limb (Gordon-Evans, 2012; Gillette, 2008). Commonly, kinetic gait analysis provides a non-invasive method of collecting gait information and is collected by using one force plate during a single stance-swing phase (Budsberg, Vertraete, Souta-Little, 1987). Kinetic gait data is collected when a dog steps onto the force plate (Strasser, Peham and Bockstahler, 2014) which exerts the magnitude of force applied by a single step and measured by the defection of the sensing elements within the plate (McLaughlin, 2001; Strasser, Peham and Bockstahler, 2014). The displacement of this sensing element is proportional to the force exerted by the step (Strasser, Peham and Bockstahler, 2014).

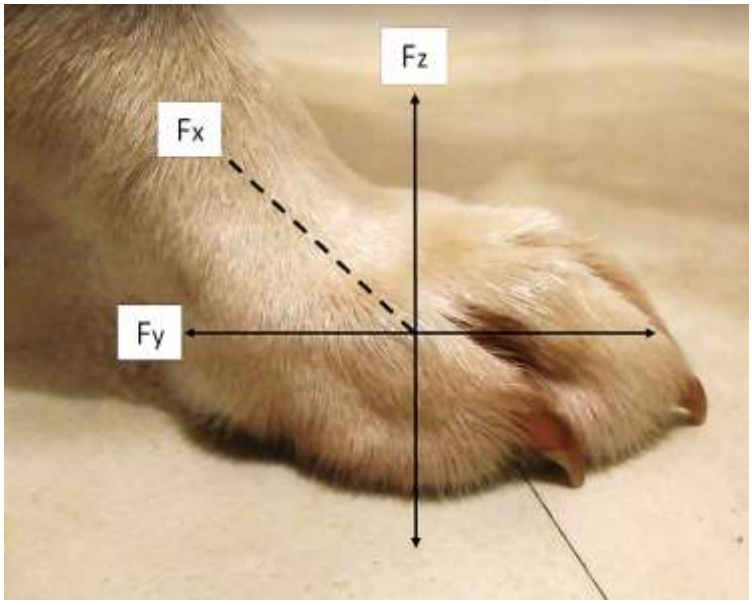

Figure 1. 2. Representation of Peak Vertical Force (PVF) in a canine foot.

Vertical force (Fz) is represented on the Z axis, craniocaudal (Fy) is highlighted by the Y axis. Mediolateral (Fx) is found on the X axis. During each step, three orthogonal ground reaction forces are calculated in three different dimensions: mediolateral (Fx), craniocaudal (Fy) and vertical (Fz) (Figure 1.2) (McLaughlin, 2001; Wustefeld-Janssens et al., 2015; Quinn et al., 2007). These measurements enable quantifiable data to be obtained for the assessment and evaluation of mobility impaired and non-mobility impaired dogs, making kinetic gait analysis more sensitive than visual observations (Evans, Horstman, Conzemius., 2005). Furthermore, additional gait evaluation can be obtained by acquiring temporal parameters for analysis such as impulses, slope and stance time which can complement ground reaction forces and provide information relating to the distribution of forces over time (Wustefeld-Janssens et al., 2015). Thus, allowing more accurate assumptions to be made when comparing mobility impaired and non-mobility impaired dogs.

For many years, a concern of using gait analysis by kinetic and spatiotemporal data was the effect of factors including body size and mass (Kano et al., 2016). However, force plate data is often normalised to the participant's body mass, thus, allowing evaluations to be made between various dog breeds and sizes (Budsberg, 1987). Additionally, it is important to take these factors into account when considering orthopaedic impairments and evaluating the success of treatment programmes (Evans et al., 2009).

It is advised that a mean of five trials are collected for meaningful statistical comparisons (Evans, Horstman, Conzemius., 2005). During a kinematic gait analysis trial, a handler leads a dog across a force plate; handlers, dogs and repetition of trials do not seem to have any effect on PVF or craniocaudal (Fy) force plate impulses (Sandberg et al., 2020). Two studies that focused on assessing gait (Bockstahler, 2004; Malikides et al., 2007) both stated that it is fundamentally important that the dog handler does not interfere with the dog's natural walking pace. Therefore, a very short lead that creates excessive tension is not recommended (Bockstahler, 2004; Malikides et al., 2007). Jevens et al (1993) investigated the percentage variance that handlers, dogs and repetitive trials had on data collected: handlers accounted for between 0-7%, whereas dogs contributed 14-69%, and trial repetition from 29-85% (Jevens et al., 1993). Therefore, it is advised where possible to keep to the same dog handler, however, it is not detrimental to the results if this is required to change during the investigation, although would depend on the research question being asked.

Two types of gait have been used in veterinary studies, walking and trotting (Walton et al., 2013, Hercock et al., 2009; Bockstahler, 2004; Malikides et al., 2007). However, trotting is more diagnostic when analysing dogs with mild lameness compared to walking gait (Voss, Imhof, Kaester et al., 2006). A study conducted on non-mobility impaired Greyhounds by Roush and McLaughlin in 1994, highlighted significant differences in peak vertical force and vertical impulse when velocity was altered during trot. Although, in severe cases of lameness, where no pain relief is given, the participant may not be able to trot, therefore, the researcher must decide on what velocity will give them the most reproducible results overall (Roush and McLaughlin, 1994). A similar study undertaken by Renberg (1999) using mobility impaired Greyhounds concluded that stance time and velocity as a control variable has no significant difference in ground reaction force. Perhaps, this is due to mobility impaired animals unevenly loading their weight (Budsberg, 1995). It has been suggested that mobility impaired dogs load weight onto the affected limb slower and subsequently, unload faster, shortening the stance time in the lame leg (Budsberg, 1995). This conclusion was made based on the relationship between stance time and velocity: as participant velocity decreases, relative stance time increases.

Greyhounds are commonly used in gait analysis due to them being defined as having “normal canine gait characterisation” (McLaughlin, 1994; Rumph, 1999; Roush, 1994). However, many canine characteristics can affect data collected, for example, body mass, angulation of limbs, the centre of gravity, frequency of strides, and whether the dog has a natural walk or trot gait (Rumph, 1999). Budsberg (1987), stated that of all the different morphometric characterisations, body mass had the best correlation to ground reaction forces and impulses exerted. In the same study, Budsberg (1987) suggested that although there are breed differences in stride frequency and length, when ground reaction forces were correlated to body mass, the measurable differences between breeds diminished. This statement was further supported by Bertram et al (2000), when trotting gait of Greyhounds and Labrador retrievers were compared and found that the two different breeds moved dynamically similar (Bertram et al., 2000). When analysing canine gait, it is acceptable to compare different breeds within the same study, as long as ground reaction forces are normalised to body mass (Bertram et al., 2000; Budsberg 1987; Sandberg et al., 2020).

The major disadvantage of kinetic gait data using single force plate systems is the inability to measure consecutive strides during a trial (DeCamp, 1997). Therefore, information surrounding the swing phase cannot be attained (Besancon, 2003). Kinematic gait analysis allows a complete analysis of the stance-swing phase even if only one force plate is present (McLaughlin, 2001). Kinematic gait analysis can provide information regarding thoracic and pelvic limbs that can be correlated with the force exerted on the force plate(s) (Budsberg, 1999).

Although kinetic gait analysis cannot serve as a clinical diagnostic test for specific pathologies, it is an invaluable tool to research into orthopaedic diseases (Colborne, 2008; Fanchon and Grandjean, 2007). To date, numerous veterinary papers have been published to describe the alterations in ground reaction forces that occur in specific orthopaedic diseases (Sandberg et al., 2020; Walton et al., 2013; Hercock et al., 2009; Kim et al., 2017), particularly, cranial cruciate ligament rupture, hip and elbow dysplasia have dominated (Böddeker et al., 2012; Evans et al., 2005; Tinga et al., 2018). Dogs suffering with from cranial cruciate ligament rupture have apparent weight-bearing lameness which can be detected on physical examination (Böddeker et al., 2012). However, ground reaction forces are also reduced due to insufficiencies in braking and propulsion forces (DeCamp, 1997). Kinetic gait analysis also provides analysis for improvement statistics following surgical correction (Amimoto et al., 2019; Tinga et al., 2018). To date, studies continue to be published that focus on the long-term improvement of lameness following surgical correction. For example, Amimoto et al (2019), evaluated the recovery of limb function by using kinetic gait analysis after tibial plateau levelling osteotomy (TPLO). Similarly, Boddeker et al (2012) used kinetic measurements to assess the degree of lameness expressed as PVF following a tibial plateau levelling osteotomy (TPLO) versus the lateral suture approach. This study concluded that the TPLO method leads to faster recovery and improved limb function four months after surgery compared to the lateral suture method (19% compared to 5.8% respectively) (Böddeker et al., 2012).

Many dogs also suffer from hip dysplasia; however, many demonstrate little lameness on physical examination (Lopez and Schachner, 2015). Escobar et al (2017) investigated 30 English Bulldogs (a breed commonly predisposed to hip dysplasia) who underwent kinetic gait analysis alongside pelvic radiographs and veterinary clinical examination to assess

locomotion. Peak vertical forces, vertical impulses, stance phase, symmetry index and rate of loading of hindlimbs were analysed. The results of this study showed that although none of the thirty dogs showed the clinical signs of orthopaedic pain, all dogs had evidence of hip dysplasia on radiography and a reduction in peak vertical force and impulse (Escobar et al., 2017). Furthermore, all of the English Bulldogs showed average pelvic limb symmetry indices of 19.8%, whereas healthy dogs are expected to have symmetry indices of close to 0% (Volstad et al., 2017). This study highlights that the English bulldogs had pelvic limb gait dysfunction consistent with hip joint disease/dysplasia causing uneven weight-bearing which was not found on clinical examination. In canine hip dysplasia, kinematic evaluation has found that there is an increase in stride length, and decreased stride width, subsequently causing a 'pelvic waddle' where there is lateral pelvic movement and mediolateral movement in the hind feet (Edge-Hughes, 2007). However, although kinetic evaluation is more readily available, it can sometimes cause veterinary researchers to miss vital information when determining the severity of progressive diseases (Edge-Hughes, 2007).

There are limitations associated with the use of quantitative gait analysis in veterinary medicine. Although minimal equipment is required to acquire kinetic gait data, this equipment is costly and potentially impractical for purchase in clinical practice (Anderson et al., 2018). Additionally, there is a need for consistent velocity, therefore, a relatively long walkway and multiple trials are required to gait accurate data, thus, use may be limited in small clinics (Jevens et al., 1993). Finally, kinetic data does not record measurements relating to stride or step length, or joint angles (Budsberg, 1999), therefore, other methods of gait data collection may be required for a detailed analysis of mobility (Sandberg et al., 2017).

1.2.3 *Kinematic Gait Analysis*

Kinematic gait analysis is often combined with kinetic gait analysis, due to both systems providing complementary information regarding canine gait (Bockstahler et al., 2007). Motion capture cameras are used for non-invasive kinematic data collection, and capture acquisition speeds of between 60 to 100Hz as this is deemed sufficient enough to capture most canine gait events (Sandberg et al., 2017). Although, there may be some discrepancies during the

gallop phase of canine gait as this requires a higher frequency (DeCamp, 1997). It is important to note that equipment recording speeds can impact gait recordings significantly, as the ability to record gait data at higher speeds improve joint and limb mobility definition (Fahie et al, 2018), especially during periods of maximal change where the greatest flexion and extension is occurring (Fahie et al, 2018).

1.2.3.1 *Two- and Three-Dimensional Systems*

Motion capture systems require all cameras to be synchronised and calibrated before data collection (Sandberg et al., 2020). Both 2- and 3D systems are in place to record kinematic gait analysis (Kim et al., 2017), however, 2D systems use a single camera and simple calibration techniques (Sandberg et al., 2020). For both 2- and 3D systems, markers are used at various anatomical landmarks (Miró et al., 2008); for 2D systems, high contrast markers which are tracked by standard video operating systems are used. (Miró et al., 2008). For 3D analysis, retro-reflective markers are tracked by specialist motion capture cameras (Sandberg et al., 2020). For 2D systems, calibration is relatively simple by measuring a known length within a desired orthogonal plane relative to the camera, a plane-of-motion can be achieved which enables any movement (via tracked markers) to be accurately measured within the specified calibrated plane (Sandberg et al., 2020). Motion capture for a 3D area is somewhat more complicated since a field-view for all cameras is required (Sandberg et al., 2020). Therefore, the use of a calibration frame and wand is paramount; the frame routinely consists of multiple markers positioned at accurate locations to represent the x, y and z coordinates, the frame is then positioned in the centre of the recording space to be detected by all cameras (McLaughlin, 2001). It is recommended that each system is re-calibrated for each use to ensure accurate data collection (Sandberg et al., 2020).

2D kinematic gait analysis is considered relatively cheap and easy to use, as only the use of a non-speciated video camera and high contrast markers are required (Atkins et al., 2013). A common limitation of 2D recording systems is that they require very strict data collection protocols (Sandberg et al., 2020). For example, accurate camera positioning is imperative to have accurate calibration and to avoid parallax and perspective error, which occurs when the animal faces away from the optical axis of the camera (either inwards or outwards) (Sandberg

et al., 2020). In contrast, 3D kinematic gait analysis is considered the gold standard (Sandberg et al., 2020). Nonetheless, there is a requirement for highly specialised equipment and laboratories to collect, process and analysis the data which often provide financial constraints (Kirtley, 2006).

2D systems cannot record movements that occur outside the calibrated area, this means there is a lack of recordings relating to abduction/adduction and internal/external rotation (Sandberg et al., 2020). In 2D (and early 3D) analysis, 'link-linear models' are commonly used to define the sagittal plane motion, mainly because of their ease of use and refined methodology in veterinary clinical research (Atkins et al., 2013). Link-linear models provide information on movement in a sagittal plane due to markers being placed at specific locations (e.g. stifle, elbow, tarsus, carpus) to allow flexion/extension movements to be analysed and body segments to be created in a linear fashion (Gillette and Angle, 2008; Jarvis et al., 2013; McLaughlin, 2001).

1.2.3.2 *Retro-reflective Markers*

A Joint Coordinate System (JCS) is commonly used in 3D investigations to enable data collection on rotation (internal/external), flexion/extension and adduction/abduction (Sandberg et al., 2020). JCS is achieved by using the six degrees of freedom, whereby three or more markers are placed in closer proximity (although not in a straight line) around a target joint to analyse a variety of movements around the target joint to define a body segment (Torres., 2010). Furthermore, the use of JCS allows researchers to translate complex joint movements into clinically relevant terms, for example, adduction and external rotation (Lin et al., 2020).

During a study by Foss et al (2013), thirty-two markers were spread a minimum of 4cm apart on Doberman Pinschers with and without Cervical Spondylomyelopathy to identify differences in gait patterns. The spacings of the markers were important as they used light-

emitting diodes (LEDs) to create a high contrast between marker and background (Pascual, 2003), thereby, ensuring the computer system did not process the markers in the wrong anatomical location. LEDs are commonly used in active optical systems (Foss et al., 2013). However, each LED is wired to a control unit which must move with/or be within the cable range of the animal during its movements (Lin et al., 2020). Passive systems rely on infrared, and the use of wireless retro-reflective markers negates the constraints of the active systems (DeCamp, 1997; Gillette and Angle, 2008). However, as all retro-reflective markers are visible et all times, manual labelling is required to ensure the markers are identified correctly, which can be especially difficult if the marker locations are close to each other (Pascual, 2003).

Marker visibility is another common challenge in veterinary gait data collection Sandberg et al., 2020. Medial markers are placed along limb segments to capture axial rotation (DeCamp, 1997). In quadrupeds, medially placed markers are often obscured by fur or the contralateral limb, therefore, a majority of medial markers are often converted into virtual markers (Gillette and Angle, 2008). Furthermore, inaccurate marker placement along a body segment can also lead to kinematic error (Kim, 2008). A study by Torres et al (2011) investigated the impact of incorrect marker position on canine gait analysis, they found that sagittal flexion and extension angles differed dramatically due to inconsistent marker positioning. Another common limitation in the use of markers are the difference in shape/size of dogs and their subsequent limb segments, between and within breeds (Sandberg et al., 2020). Therefore, as described above, Greyhounds (or similar large breeds) are commonly used in gait studies to reduce the impact of different morphology and soft tissue artefacts (Hottinger et al., 1996; Korvick et al., 1994).

1.2.3.2.1 Artefacts

Another major source of marker error in gait analysis is the presence of soft tissue artefacts (Lin et al., 2020). Soft tissue artefacts often occur due to the displacement of the skin surface in relation to the underlying musculoskeletal structures during movement, commonly during flexion/extension, thus, decreasing the accuracy of the spatiotemporal data collected (Lin et al., 2020). Whilst complicated mathematical filters can reduce the impact of these inaccuracies, they are still an obstacle veterinary researcher are trying to overcome. Invasive

kinematic data collection offers an opportunity to reduce the impact of soft tissue artefacts, however, this technique is highly invasive and their use remains in a non-clinical setting (DeCamp, 1997). Electrogonimometry (non-invasive) has been used in multiple veterinary studies (Gjeltema et al., 2018; Thomas et al., 2006; Colin et al., 2006) and can detect gait movement without the need for surgical attachment to the skeleton, yet, adequate connection to skin proves difficult in veterinary patients and creates soft tissue movement artefacts (DeCamp et al., 1996; Gjeltema et al., 2018). Therefore, their use is minimal and not practical within a veterinary setting (Nunamaker and Newton, 1985; Thomas et al., 2006). Non-invasive, computer-generated kinematic gait recording (either two- or three-dimensional) using motion capture cameras has proven the most effective method of collecting canine gait data (Sandberg et al., 2020). Similarly, biplanar X-ray is considered the most accurate method to gain skin motion via free-motion data collection, the method has been applied to the dog by Fischer et al (2018).

1.2.3.3 *Application of Kinematic Gait Analysis in Veterinary Research*

In recent years, kinematic data collection has been used alongside kinetic gait analysis to provide more information about prognostic factors pre-and post-surgical intervention (Alam et al., 2011). In canine cranial cruciate ligament injuries, kinematic gait analysis has helped to characterise joint-specific lameness in the pelvic limb. Early studies (Vilensky et al., 1994; DeCamp et al., 1996) found that the femorotibial joints of dogs suffering from cranial cruciate ligament rupture remained more flexed throughout a stride event, particularly during late stance and early swing phases where propulsion of the limb is exacerbated. Tinga et al (2018), found that non-disease femorotibial joints have greater flexion during the gait cycle than the diseased joint. Research into the effects of chronic cranial cruciate ligament rupture on the femorotibial joint shows that although surgical correction is desired, and kinematic analysis six months post-surgery showed improved lameness, dogs will never return to pre-injury levels (Farese et al., 2005) suggesting that kinematic gait analysis of pelvic limb.

The use of kinematic gait analysis in veterinary orthopaedic research has also proven extremely beneficial (Walton et al., 2013; Sandberg et al., 2020). However, these studies have required careful planning and have been conducted in controlled environments (Walton et al., 2013, Hercock et al., 2009). Research using kinematic gait analysis in veterinary species has progressed indefinitely and whilst 2D analysis is still most commonly used, the expansion of specialist centres that can facilitate 3D kinematic gait analysis will allow more efficient data collection for extrapolation into clinical settings (Sandberg et al., 2020).

1.3 The Use of Clinical Metrology Instruments (CMIs) in Veterinary Medicine

Since kinetic and kinematic systems are not routinely found in clinical settings, other methods of determining mobility, such as owner-reported questionnaires, can be used to assess and diagnose specific impairments. Although these questionnaires may not be as sensitive to changes in PVF or VI, their use should not be dismissed. Questionnaires that evaluate psychometric properties (reliability, validity and norming) are commonly used in human medicine (McEnvoy et al., 2010; Shyu et al., 2006; Mann, 1985). Psychometric testing provides data that measures specific outcomes on mental health, mobility and surgical success. For this reason, all instruments should be standardised, validated and reliable to avoid bias (Boivin et al., 2011) and ensure the data allows for patient-orientated decision making (Cook, 2007).

Clinical Metrology Instruments (CMIs) are questionnaires aimed to evaluate and address clinically relevant questions about a specific construct, e.g. canine mobility (Wiseman-Orr et al., 2006). Orthopaedic CMIs used in human medicine include Knee injury and Osteoarthritis Outcome Score (KOOS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and have since prompted use in the veterinary profession (Muller et al., 2016). Veterinary CMIs for assessing canine OA have been validated, most commonly by comparison to similar CMIs, imaging, kinematic or kinetic gait analysis (Walton et al., 2013, Hercock et al., 2009; Hielm-Björkman et al., 2003) to provide accuracy relating to reproducibility, accuracy and reliability (McHomey and Ware, 1995; Angst et al., 2005).

1.3.1 *Clinical Metrology Instruments (CMIs)*

A study by Spofford et al (2013) investigated the use of the subjective-objective-assessment-plan (SOAP) methodology when animals were presented at a veterinary practice without any obvious clinical signs noted by the owner. The study empathised the benefit of this tool, combined with regular evaluation and assessment of 'healthy' animals, in promoting early intervention and evolution of diseases and reduced suffering, which contributed to a higher quality of life for the animal (Spofford et al., 2013). It is important to remember that dog owners are the most reliable information source about changes in their animal's demeanour or behaviour, although, in some cases, dog owners may find it hard to explain the changes they have observed (Cook, 2007); this can occasionally prevent dog owners reporting the change to their veterinary surgeon or provide the correct description when explaining the abnormality (Boivin et al., 2011). Additionally, some owners will relate changes in their animal's routines or behaviours to natural phenomena, and often attributable to old age or behavioural issues (Cook, 2007).

When assessing mobility or evaluating function, there are numerous factors that need to be taken into consideration, and therefore, may be observed from different perspectives (Hielm-Björkman et al., 2003). In most cases, the interpretation of these abnormalities is not dissimilar between dog owners and veterinary surgeons (Walton et al., 2013). Of course, the individual characteristics of a canine patient must also be taken into consideration when evaluating the causation of movement or mobility abnormalities (Hielm-Björkman et al., 2003), highlighting the importance of client information and input when making a diagnosis (Hercock et al., 2009). CMIs have been used to assess chronic pain or quality of life, using a combination of questions based on mobility, pain severity and related behaviours, lifestyle, quality of life and demeanour (Muller et al., 2016), these individual item scores are then used to calculate an overall instrument score (Walton et al, 2013; Cleeland and Ryan, 1994). Client-Specific Outcome Measures (CSOM) are considered more specific to the individual and are based on distinct activities which the owner feels are problematic for their dog (Muller et al., 2016).

A validated CMI helps to minimise confounding variables and provide more accurate results (Hercock et al., 2009). Furthermore, most CMIs are commonly used in conjunction with body

condition scores, vital signs, clinical examinations and gait analysis (Hesbach, 2007; Millis, 2014), hence, there is a reduced inference from the truth. Most studies have shown good repeatability for validated CMIs when used to assess pain and mobility (Walton, et al., 2013; Brown et al., 2013).

1.3.2 *Assessment of Mobility Impairments with CMIs*

In both human and veterinary settings, mobility and activity are concepts that are closely related to the state of health, well-being and quality of life (Millis, 2014) of the patient. . Conversely, companion animal functional mobility lacks specific attention, as there are few individual domains with fully validated instruments developed (Millis, 2014). Instead, studies have focused on associations with signs of disease and clinical features to evaluate pain and quality of life (Hielm-Bjorkman et al., 2003; Brown et al., 2007; Holton et al., 2001). Musculoskeletal disorders in companion animals have been widely studied, however, no study has fully characterised "normality" (Walton et al., 2013). The limited investigation into healthy dogs alone is somewhat a critical issue, especially for determining the early onset of mobility problems (Walton et al., 2013) and how they are recognised since this can help provide a preventative approach and identify a sub-clinical state (DeCamp, 1999).

There are approximately six CMIs used to determine orthopaedic pain in dogs, with four commonly used in clinical practice (Walton et al., 2013), including Liverpool Osteoarthritis in Dogs (LOAD), Helsinki Chronic Pain Index (HCPI), Canine Orthopaedic Index (COI) and Canine Brief Pain Inventory (CBPI). These CMIs are commonly used in veterinary practices due to their open-access availability and accessibility for use in numerous musculoskeletal conditions (Muller et al., 2016). The LOAD is used to assess articular orthopaedic disorders and was first validated to evaluate canine elbow osteoarthritis (Hercock et al., 2009). Previous studies have shown that the LOAD correlates favourably ($r_2 = 0.74$) with the Canine Brief Pain Inventory and with objective measures of mobility such as gait analysis ($r_2 = 0.23$) (Walton et al., 2013). The LOAD questionnaire asks dog owners to assess their dogs' mobility in thirteen different areas (five general domains and eight domains relating to exercise), scores are placed on a 0-4 simple descriptive scale (SDS). The Helsinki Chronic Pain Index (HCPI) assesses chronic pain

associated with canine OA using a 0-4 Likert scale to assess 11 different items. The HCPI was first developed in 2003 (Hielm-Björkman et al., 2003), and fully validated by dog owners completing the questionnaire 5 times during a 16-week period in 2009 (Hielm-Björkman et al., 2009a). The HCPI has been frequently used to assess the therapeutic response to treatment for OA (Hielm-Björkman et al., 2009a; Hielm-Björkman et al., 2009). One recent study (Okamoto-Okubo et al., 2021) reported HCPI correlated well with objective gait analysis when assessing response to treatment (scores decreased by 41%, 52%, 51%, and 48%, respectively over 60 days).

The Canine Brief Pain Index (CBPI) was initially adapted from a commonly used human CMI (Cleeland and Ryan, 1994; Brown et al., 2009) and has since been validated for use in canine OA in 2007 by comparing questionnaires responses between OA and non-OA groups (Cimino-Brown et al., 2007; Brown et al., 2008; Sullivan et al., 2013) and osteosarcoma in 2009 (Brown et al., 2009; Brown et al., 2013). Similarly, the HCPI measures 11 different items, with domains varying from pain severity (0-10), pain interference with function (0-10) and quality of life (0-5). CBPI has also been used to assess response to therapy for canine osteoarthritis (Brown et al., 2008; Sullivan et al., 2013), and in 2013 correlated with objective measurements of canine osteoarthritis (Brown et al., 2013). The Canine Orthopaedic Index (COI) is a validated tool created was created by the American College of Veterinary Surgeons (ACVS) with the aim to quantify the quality of life (QoL) in dogs suffering from orthopaedic conditions. Its use is widespread (Cimino-Brown, 2014; Alves et al., 2020). It has recently been translated into Swedish to evaluate the validity, reliability and sensitivity of the questionnaire in dogs with elbow dysplasia (Anderson and Bergström, 2019). The structure of COI is similar to CBPI, whereby, 11 items are investigated. The COI uses the NRS of 0-5 instead of 0-10, potentially hindering its application for outcome-based medicine in veterinary orthopaedic assessment (Brunelli et al., 2010).

In addition, multiple CMIs exist for owners to complete for other health conditions including: Canine Behaviour Questionnaire, Health-related Quality of Life questionnaire and University of Glasgow Nutrition Questionnaire (Anderson and Bergström, 2019). These CMIs allow assessment of physical health, alongside giving owners and veterinary surgeons a sense of physical and emotional discomfort, and management of stress within animals, alongside highlighting the importance of preventative care to maintain health and welfare (Alves et al.,

2020). Studies have also shown the use of CMIs in veterinary practices can improve communication between veterinary surgeons and dog owners (Anderson and Bergström, 2019; Muller et al., 2016).

1.3.3 *Validating Clinical Metrology Instruments (CMIs)*

After the newly developed questionnaire has undergone preliminary pilot testing and revisions, pilot studies must be conducted for validation of the CMI for use in clinical settings (Connell et al., 2018). The validity of a questionnaire is determined by analysing whether the questionnaire measures what it is intended to measure; validity is subdivided into four categories: Content, Face, Construct, and Criterion (Connell et al., 2018; Mousazadeh et al., 2017).

Content validity refers to the extent to which the instrument measures the appropriate content (Walton et al., 2013). It also provides information about the variety of attributes that make up the desired construct, and if they are relevant and accurate (Frost et al., 2007). Typically focus groups are used to determine content validity by methods used in scale construction to identify important variables, and select items which are necessary for the CMI (Connell et al., 2018), subsequently leading to the inclusion or omission of items within the questionnaire (Dean, 2015). **Face validity** is similar to content validity in that it refers to the extent to which the items within the questionnaire measure the intended construct (Frost et al., 2007). Face validity is usually tested through a review process, whereby, experts in the field review the questionnaire to ensure the construct is being tested- (Frost et al., 2007). **Construct validity** investigates how well the construct of the newly developed instrument matches the true construct that is being measured and reflects the hypothesis (Frost et al., 2007). Commonly, construct validity is tested by administering the CMI to two groups of varying clinical status (for example, those with and without mobility impairments). Comparing the results to highlight how the construct correlates with similar measures, and how it differs (Walton et al., 2013). Various studies have tested construct validity by testing the newly designed instrument against a current validated CMI testing the same construct (McEnvoy et al., 2010). Walton et al (2013) tested the LOAD questionnaire against other similar measures

to provide evidence of construct validity. LOAD, CBPI and HCPI CMI scores were compared against each other to determine the construct validity; factor analysis was used to reduce data into simplified components for analysis, so they could be explained by the theoretical construct underlying the LOAD instrument. Results from this study showed there were significant moderate correlations between LOAD, CBPI and HCPI CMIs, suggesting there is construct validity for all of the above instruments. Similarly, Muller et al (2016) conducted a study to evaluate CMIs in dogs with osteoarthritis. The CBPI, LOAD, HCPI and Client-Specific Outcome Measures (CSOM) were compared to determine construct validity and variability between CMIs used to assess pain in dogs suffering mobility impairments. Owners of fifty-one dogs with osteoarthritis completed all four veterinary CMIs. Results showed a good agreement between baseline CMI scores at various time points within the investigation (Muller et al., 2016). The LOAD questionnaire had the best agreement and gave the most consistent results over the investigation period. However, there was no significant difference between the average recorded values for all four CMIs, suggesting, CMIs are of great benefit to veterinary practitioners as they are reliable, and thus, prove very useful in a preventative medicine perspective by detecting early, subtle signs of illness (Walton et al., 2013; Muller et al., 2016).

Finally, **criterion validity** refers to the extent of which the newly designed instrument correlates with a standard, external measure. In human and veterinary mobility studies, the newly developed CMI is compared against the gold standard way of determining the construct, most commonly, kinetic gait analysis. Walton et al (2013) used kinetic gait analysis on 222 dogs to gain data on ground reaction forces (specifically peak vertical force (PVF)) to test correlations between CMI scores (LOAD, HCPI and CBPI instruments were used) and PVF symmetry scores. The results of this study showed that there were significantly weak correlations between LOAD and CBPI scores and PVF symmetry scores. It is not surprising the correlations between these scores are weak, however, since dogs with bilateral disease may have impaired but symmetrical mobility (Walton et al. 2013). Conversely, dogs with marked lameness on one joint may have overall good mobility, but marked asymmetry in comparison with three other healthy joints (Radke, Joeris and Chen, 2021). Similarly, Hercock et al (2009) used 26 dogs with chronic elbow osteoarthritis to determine the validity (face and criterion) of the LOAD questionnaire; criterion validity was assessed by comparing aggregate scores of

the LOAD questionnaire and PVF at the same visit using Spearman's rank correlation coefficients. However, in contrast to the above study by Walton et al (2013), the LOAD and PVF scores were poorly correlated, suggesting a mismatch between PVF and client perceptions of lameness. Although, it is important to note that there is evidence suggesting that PVF decreases as dogs become more lame (Cross et al., 1997; Waxman et al., 2008). Furthermore, owners may be classifying 'lameness' differently compared to a veterinary surgeon's definition of lameness. Additionally, as mentioned above, PVF and lameness do not have a linear relationship due to visual gait/lameness assessment being subjective (Waxman, Robinson, Evans et al., 2006). Therefore, although Hercock et al (2009) found there is a poor correlation between LOAD scores and PVF symmetry scores, this may be due to the LOAD questionnaire capturing different valuable information relating to mobility other than PVF.

CMIs have been used to assess chronic pain or quality of life by combining questions relating to pain severity, behavioural changes, quality of life and demeanour (Muller et al., 2016). However, CMIs are not without subjectivity, predominantly due to the caregiver placebo effect (owners incorrectly perceive improvement/deterioration in their pets' health) (Gruen, Dorman and Lascelles, 2017). Conzemius and Evans (2012) found caregiver placebo effect occurred 39.7% of the time when owners were asked to evaluate their dog's lameness, thus, objective measures of lameness (i.e. gait analysis) should be used to give unbiased results (Conzemius and Evans, 2012). Where gait analysis is not available, CMI scores should be used in conjunction with body condition scores, veterinary clinical examination and visual gait assessment to gather information regarding functional strength, motor control, static and dynamic balance and proprioception (Hesbach, 2007; Millis, 2014). Some studies have shown good repeatability for validated veterinary orthopaedic CMIs when used to assess pain and mobility (Walton, et al., 2013; Brown et al., 2013) when discontinuous ordinal scales or visual analogue scales are used.

The above literature suggests there is beneficial use for veterinary clinical examination and veterinary CMIs when detecting canine mobility impairments. However, their use is occasionally questionable when not combined with more sensitive methods of canine gait analysis, such as kinetic or kinematic assessment. Similarly, most pre-validated owner-reported questionnaires aim to provide mobility assessments for dogs who have chronic orthopaedic conditions, whereas the current project intends to validate a new owner-

reported mobility questionnaire (GenPup-M) which aims to detect all mobility impairments, including any subtler, early changes.

Similar to Walton et al (2013), Principal Component Analysis (PCA) will be used to identify specific components of the GenPup-M questionnaire which enable the detection of mobility impairments. The LOAD questionnaire (previously validated by Walton et al., 2013) will be used to provide criterion validity for GenPup-M. Due to time restraints of this project, a validated veterinary orthopaedic clinical examination sheet (Harris et al., 2018) will be used to categorise dogs into mobility and non-mobility impaired cohorts, and will therefore be used as the gold-standard objective measure of gait assessment. Additionally, since mobility impairments are being assessed, the validated clinical examination will provide data relating to the transitions between lying down and standing up phases, pain associated with manipulation of joints, and ROM. Quantitative gait analysis (kinetic and kinematic gait analysis) will be used to provide construct validity for GenPup-M. It is hoped the above methodology will allow validation of GenPup-M as the first owner-reported canine mobility tool to detect all canine mobility impairments. Once validated, GenPup-M can be used routinely in veterinary clinical settings to aid the longitudinal assessment of a dog's mobility.

1.4 Hypothesis

The hypothesis tested, and reported in this thesis, is that the GenPup-M questionnaire will be able to identify early mobility differences in a cohort of dogs who have undergone a full veterinary orthopaedic and neurological clinical examination and kinetic and kinematic gait analysis. We also hypothesize that the GenPup-M questionnaire will be able to correctly identify mobility impairments that were identified via quantitative canine gait analysis or a validated clinical examination.

1.5 Study Aim

The research reported within this thesis aimed to use independent veterinary assessments and gait analysis to validate a set of questions ("GenPup-M") that were designed to collect owner-reported canine mobility data in dogs with/without mobility problems. Validation of GenPup-M will allow detection of all canine mobility impairments (including early-onset impairments) for use in clinical veterinary practices to improve canine health and welfare.

1.6 Study Objectives

1. Collect owner-reported data using a novel mobility questionnaire ("GenPup-M") from dogs presenting to the first opinion and referral small animal veterinary hospitals at the University of Liverpool.
2. Obtain veterinary assessments and quantitative gait data (using 3-D motion capture and force plates) on dog groups detailed in Objective 1.
3. Compare data collected in Objectives 1 and 2, in order to assess the accuracy of owner-reported canine mobility data collected as part of this study (Objective 1)
4. Determine whether individual (within dog analysis) mobility impaired and non-mobility impaired dogs differed in kinetic and spatiotemporal parameters, compare these data to see if these changes are consistent across the categories (between dog analysis).
5. Produce a validated questionnaire tool, based on results from Objective 3, for use within research studies within small animal veterinary practices, to identify early mobility issues.

1.7 Ethical Approval

The study received ethical approval from the Veterinary Research Ethics Committee (VREC), Institute of Veterinary Science, University of Liverpool, reference number VREC942. An additional COVID-19 exemption request form was also submitted for ethical approval to ensure the safety of the investigators and participants for the duration of this study (VREC942a).

The methodology involved no invasive or stressful events for the dog, fresh water was always available in the gait laboratory. The dog owners gave informed consent prior to the dog participating in the study.

CHAPTER TWO | GENPUP-M QUESTIONNAIRE

2.1 Background

Clinical Metrology Instruments (CMIs) such as GenPup-M have been used within veterinary practices to aid clinical decision-making and assess the long-term deterioration of health or welfare (Angst et al., 2005). However, their use is not entirely standalone for mobility assessment, and is often combined with various 'gold standard' approaches to assess the extent of the condition.

Eithne Comerford (EC) worked closely with the Dogs Trust 'Generation Pup' team (including Dr. Jane Murray (JM), Rachel Casey, Rachel Kinsman, Severine Tasker, Michelle Lord and Toby Knowles) to develop a mobility questionnaire which could collect mobility data from dog owners at repeated time points using the Generation Pup longitudinal study. The GenPup-M questionnaire is currently being used to collect mobility data from dog owners at repeated time points (from age 5 months) as part of their longitudinal monitoring within the Generation Pup project (Murray et al., 2021). However, objective validation of GenPup-M has not been performed for use in either Generation Pup or the general dog population. Questionnaire validation is essential before detailed analysis and publication of risk factors for owner-reported canine mobility problems can be undertaken. Validation of questionnaire data provides reassurance that GenPup-M can accurately identify subtle changes relating to canine mobility reported by dog owners. Furthermore, the identification of risk factors indicative of early reductions in mobility can be used to develop future interventions, thus, improving canine welfare.

Multiple veterinary studies have tried to determine canine mobility impairments via CMIs and objective gait analysis (Muller et al., 2016; Walton et al., 2013; Hercock et al., 2009; Hielm-Björkman et al., 2003). In some instances, CMIs and gait analysis have positively correlated (Walton et al., 2013; Hercock et al., 2009), in others, this correlation has been weak, as discussed in Section 1.4.1. These positively weak correlations could be explained by the numerous factors which must be taken into consideration when assessing canine mobility (Hielm-Björkman et al., 2003), including temperament and disposition of the dog,

conformational abnormalities or duration of the mobility impairment (Angst et al., 2005) However, many veterinary CMIs for clinical use have been validated by the use of kinetic, kinematic, imaging and other objective measures of outcome (Cook, 2007). Furthermore, not all veterinary practitioners have access to sophisticated instruments to measure gait analysis, hence, having a tool which is sensitive to detect early behavioural or lifestyle changes which are indicative of decreased mobility, is beneficial in creating treatment plans (Anderson et al., 2018).

2.2 Materials and Methods

2.2.1 *Sample Size Calculation*

An online sample size calculator (sampsize.net) was used to estimate the required sample size to compare aggregate GenPup-M scores between two groups of dogs: those showing a) no clinical signs and b) clinical signs of mobility impairment following a validated clinical veterinary examination. It was estimated that in order to detect an average difference between the two groups of 10 points (10 points were judged to be the difference between mild and moderate lameness). A sample size of 23 dogs per group (power = 0.8, significance level = 0.05) was required. To account for possible participant attrition, a target sample size of 30 dogs per group was required. This number was deemed a feasible target given the time and resources available for this Masters project.

2.2.2 *Inclusion and Exclusion Criteria*

The **inclusion criteria** consisted of the enrolment of all dogs over five months of age in the study if the consulting veterinary surgeon Natasha Clark (NC) and the owner had no concerns about the dog being subject to an independent veterinary assessment for mobility and gait analysis, based on health and/or behaviour concerns.

Exclusion criteria: Any dogs which were receiving medication (for example non-steroidal anti-inflammatories (NSAIDs)) for pain relief for mobility or other health-related problems, were excluded from the study to prevent pain relief masking mobility problems that would otherwise be expected to be evident on veterinary examination or from objective gait analysis assessments. However, any dogs receiving nutraceuticals were included in the study as it has been previously suggested that this would not disguise any mobility impairments (Walton et al., 2013; Vandeweerd et al., 2012). Specific questions regarding if dogs were receiving any medication, which could potentially hide mobility impairments, were included in the GenPup-M questionnaire.

2.2.3 *Recruitment of Canine Participants*

All dogs who participated in this study were initially going to be recruited from owners of dogs presented for routine appointments at the University of Liverpool (UoL)'s Small Animal Practice and the University of Liverpool's Small Animal Teaching Hospital. However, the methodology to recruit participants was changed early in the project due to government restrictions for the COVID-19 pandemic. No dog owners and only essential veterinary surgeons were allowed in the Small Animal Practice (SAP) and Small Animal Teaching Hospital (SATH) between September 2020 and April 2021. Thus, no participants could be recruited for the project via this route. Advertisements for canine study participants for this project were sent to staff and student members within the Institute of Life Course and Medical Sciences at the University of Liverpool. Initially, the gait and clinical examination data were meant to be recorded using the purpose-built gait laboratories based within the William Henry Duncan (WHD) building, Institute of Life Course and Medical Sciences, and Leahurst Campus,

University of Liverpool. However, due to COVID-19 restrictions, all data collection was moved to be solely based at the William Henry Duncan building for the duration of the project.

Owners willing for their dogs to participate in this study were asked to contact the Primary Investigator EC or NC. If the dog fulfilled the inclusion criteria, he/she could be enrolled for data collection. Prior to data collection, the participant ID number, age and breed of the dog, along with the clients contact details were recorded using an Excel 2019 (Microsoft®) spreadsheet. All personal data relating to this project was pseudonymised and kept on a password-protected University drive which was only accessible by EC and NC.

Once the dog owners were recruited onto this study, they were provided with a written information sheet (Supplementary Material 1) summarising the research procedure and were given the opportunity to ask questions about the study). The questionnaire was initially intended to be completed at the time of the clinical examination and gait analysis, however, COVID-19 restrictions in place at the time of participant recruitment prevented the owners from being present during data collection. NC arranged a Microsoft Teams (Microsoft®, 2020) call/meeting for an informal chat with the owner(s) approximately seven days before the date agreed for data collection to convey the above information. Owners were then asked to provide online written consent to participate; consent forms were signed at the same time the owners brought their dog(s) into the WHD building for data collection. The consent forms were then collected and recorded on the University's dedicated SharePoint site to which only EC and NC had access.

2.2.4 *Comparison to Liverpool Osteoarthritis Questionnaire in Dogs (LOAD)*

The LOAD questionnaire was included in this study to assess criterion validity for GenPup-M. LOAD is frequently used within clinical veterinary settings and has been used in multiple veterinary research projects to assess canine mobility (Walton et al., 2013; Chiu et al., 2020; Roberts et al., 2021). Further rationale for use of the LOAD questionnaire, alongside validation techniques can be found in Chapter Five, Section 5.2.4. During this project, owners were asked to complete the GenPup-M questionnaire before the LOAD questionnaire. This was to ensure that the owners would read the questions carefully and not predict answer options,

owners were not biased by similar questions contained within the LOAD questionnaire and to ensure the same methodology as Muller et al., 2016. However, previous questionnaire design literature does not report any detrimental effects with completing questionnaires in a non-sequential manner (Bowling, 2005; Boynton and Greenhalgh, 2004; Edwards, 2010).

2.2.5 *Collection of GenPup-M Questionnaire Data*

Due to COVID-19 restrictions, dog owners were not permitted to be present during the clinical examination or gait analysis at the William Henry Duncan building. Therefore, completion of the questionnaire had to be done remotely, rather than the initial plan of owners completing the paper format (Supplementary Material 7) during quantitative gait analysis in the WHD building. The GenPup-M questionnaire was converted into an online format using an online survey tool (JISC.ac.uk) used by the University of Liverpool.

The client information sheet and consent form (Supplementary Material 1 and 2) were changed accordingly to comply with the new questionnaire format, and owners were also briefed about the change during the online meeting via Teams. The URL link, along with the participant ID number (ranging from 1 to 62) and password for the questionnaire was emailed to each owner using a University of Liverpool password-protected email account. The email was sent to the dog owners 14 days before the date agreed for data collection, a reminder email was sent to each owner two days before data collection to ensure they had completed the questionnaire. A contingency plan to have spare paper versions of the questionnaire was arranged, in case the owners had not completed the questionnaire prior to the visit.

An online version of the GenPup-M questionnaire was designed as a replica of the paper version; therefore, the questions all remained unchanged. However, an additional participant ID number text box was included for the owner to input their assigned number. The questionnaire was unidirectional, and therefore, the owners could not go back and change their responses. An extra free-text box was created at the end of the survey to provide the owners with the opportunity to input any changes to their dog's exercise regime, the author felt this was particularly important given the current COVID-19 restrictions, as this may have increased/decreased the daily exercise amount the dog was receiving, and thus, have an

impact on mobility. The questions were not marked as mandatory to complete before moving on to the next question, as it was thought this may decrease the questionnaire completion rate, however, owners were advised to complete as many questions as they could. The methodology changed slightly for the LOAD questionnaire, as the LOAD questions are specifically aimed at owners of dogs suffering from chronic mobility problems, thus, questions were not applicable for owners of non-mobility impaired dogs. In this instance, owners were advised to leave these questions blank and only complete questions which were applicable answered for their non-mobility impaired dog.

As NC and EC did not have access to the GenPup-M questionnaire responses prior to gait analysis, categorising dogs into mobility impaired and non-mobility impaired groups were achieved by clinical examination alone and not by the use of GenPup-M responses. Only dogs which met the inclusion criteria were assessed by NC and EC for either presence or absence of mobility issues, due to this, owners were asked about any medication their pet was receiving during the online meeting.

2.2.6 *Analysis of GenPup-M Questionnaire Responses*

Both GenPup-M and LOAD JISC questionnaire responses were exported from JISC.co.uk to *Microsoft (MS) Excel* (Microsoft Office 2019, MS Corporation). Once exported to MS Excel, the raw data was processed so that all data corresponding to mobility function were pseudonymised. Dog names were also removed and replaced with participant identification numbers (PIDs). Questionnaire response data was coded in the following format: Yes '1', No '0', Unsure/Unknown '-33', and Missing responses '-99'. Minus numbers were chosen as there was an assumption that these numerical values would not occur within the questionnaire dataset. Free text options were also analysed to see if the responses would have a direct impact on mobility outcomes, for example, if activity levels increased or decreased during COVID-19, or if a new dog had entered the household.

The "GenPup-M" questionnaire included ten 'scale' questions about the dog's mobility which were validated during this project; each question required the owners to provide a rating

from 0-10 (0 = not at all, 5 = moderately and 10 = extremely) on how they thought their dog's mobility was affected in the provided example (Supplementary Material 7).

2.2.6.1 Coding GenPup-M in SPSS

Numerical rating scores were recorded for the questionnaire responses and the corresponding meaning was highlighted via the 'Values' option in SPSS (IBM SPSS Statistics for Windows, Version 27.0). SPSS was used to analyse questionnaire data that were cleaned and entered into SPSS. Data were also categorised into 'Scale', 'Nominal' or 'Ordinal' data (Mishra et al., 2018). Age was considered 'Scale', Yes/No questions were coded as 'Nominal', and the ten scale/rank 0-10 questions were coded as 'Ordinal' data. After coding, GenPup-M had a total of seventy different variables (questions), and LOAD had eighty-one variables (questions). Dogs were categorised as non-mobility impaired with scores <20, mild for 20-34, moderate for 35-49 and severe for 50+, for ease of comparison against clinical examination score categories. There were no severely impaired dogs within this cohort identified by owners.

2.2.6.2 Principal Component and Factor Analysis

Previous work (Walton et al., 2013), used factor analysis to identify question weightings in order to determine if one or more of the main components predicted the absence/presence of mobility issues in the cross-sectional cohort of dogs. As described by Walton et al (2013), the ten scale/rank questions within GenPup-M were predominantly used for factor analysis. A Kaiser-Mayer-Olin measuring of sampling adequacy (>0.6) was used. Eigenvalues of >1, scree-plot analysis and theoretical interpretability enabled extracted factors to be assessed. Varimax-rotated models were used on extracted components to assess item loading of factor analysis. Communalities had a cut-off value of 0.4.

2.2.6.3 Spearman's Rank Correlation Coefficient

The untransformed variables were not normally distributed, hence, Spearman's rank correlation coefficient ($p \leq 0.05$, two-tailed) was used to explore the comparison between GenPup-M and the previously validated LOAD questionnaire scores. The LOAD questionnaire contains an option to create a tabulated overall mobility score based on owner responses, ranging from normal to severe. However, GenPup-M does not contain a scoring system, therefore, one was created for this project (ranging from 0-10) to allow construct validity to be assessed. A Mann-Whitney U test was used to determine the sensitivity of GenPup-M by comparing mobility impaired versus non-mobility impaired scores. Standard error of skewness, and subsequently, the degree of skewness was analysed to see if the data were significantly positively or negatively skewed by comparing the numerical value of "skewness" with twice the standard error of skewness for both negative and positive values.

Finally, Cronbach's Alpha test was used to determine internal consistency within the cohort for both LOAD and GenPup-M responses where acceptable = 0.7-0.79, good = 0.8-0.89, excellent 0.9-1.0 (Travakol and Dennick, 2011).

As per objective 5 (Section 1.6 "produce a validated questionnaire tool, based on results from veterinary assessments and quantitative gait data, for use within the GenPup study and small animal veterinary practice identifying early mobility issues"), GenPup-M was validated using previous methodology (Walton et al., 2013; Hercock et al., 2009; Huller et al., 2016) to meet this project objective. Construct validity was determined by using the validated LOAD questionnaire. Criterion validity was achieved by comparing aggregate GenPup-M scores against PVF and a validated clinical examination sheet (Harris et al., 2018) using Spearman's Rank Correlation Coefficient, where a coefficient >0.68 indicates a strong correlation (Taylor, 1990).

2.3 Results

2.3.1 *Questionnaire Responses*

A total of n=50 dog owners completed the GenPup-M and LOAD questionnaire for a total of n=62 dogs. There were a total of one-hundred and thirty-three 'missing' data responses to questions within the GenPup-M questionnaire and twelve 'unsure' responses across seventy individually coded questions. Missing responses were predominantly found in "how old was your dog when he/she started with mobility problems" and "which medication does your dog take". However, the latter may be due to the fact that the dog was not receiving any medication. Similarly, unsure responses most commonly arose from questions relating to previous surgeries or treatments for mobility problems. There were four-hundred and eighty-four 'missing' responses and zero 'unsure' responses for the LOAD questionnaire comprising of eighty-one separately coded questions. There were no 'not applicable' responses for the LOAD questionnaire, thus, many 'missing' responses originated from questions directly relating to mobility impaired dogs, for example, "how old, in months, was your dog when he/she was diagnosed with mobility problems" or "does your dog suffer from any other health conditions apart from their mobility problem". Similar to above, much of the missing data could be explained by questions not applying to dogs without mobility impairments. Scale and rank questions which were used for the data analysis which had no 'missing' or 'unsure' responses in either the LOAD or GenPup-M questionnaire.

Free-text answers were allowed for 'Other' options; however, these were scarcely used by owners due to the broad range of response options and the majority of free text included brand names of flea or wormer products or medication the dog was receiving for another health condition. The majority of owners (67%) stated in the free-text box that COVID-19 has allowed them to increase their dogs' exercise, with 11% saying they spent less time exercising their dog (predominantly, due to home-schooling or family commitments). Eleven owners (22%) left this option blank.

For the 10 scale and rank questions, there was a variation in scores between mild-and moderately-mobility impaired dogs; 10 points were found to be the difference between mild

(mean (SEM) and SD: 33.72 (2.03) ± 10.15) and moderate (mean (SEM) and SD: 44 (2.58) ± 6.32) mobility impairments using the GenPup-M questionnaire. Non-mobility impaired dogs yielded scores of (mean (SEM) and SD: 12.34 (3.25) ± 4.92).

2.3.2 *Principal Component Analysis | GenPup-M*

A principal component analysis (PCA) was conducted on the 10 items with orthogonal rotation (varimax). The Kaiser–Meyer–Olkin (KMO) measure verified the sampling adequacy for the analysis, KMO = 0.83 ('very good' according to Field, 2009). All KMO values for individual items were > 0.76, which is above the acceptable limit of 0.5 (Field, 2009). Bartlett's test of sphericity χ^2 (45) = 406.473, $p < 0.001$, suggested that correlations between items were sufficiently large, and therefore, conducting a PCA was favourable.

An initial analysis was run to obtain Eigenvalues for each component in the data. Two components had Eigenvalues over Kaiser's criterion of 1 (Table 2.1). Percentage variance of component 1 (46.82%) and component 2 (21.49%) created a cumulative percentage variation amounting to 68.30%.

The scree plot (Figure 2.1) was slightly ambiguous and showed inflexions that would justify retaining both components 1 and 2. There was scope to potentially include another component if the initial Eigenvalues had been reduced, this is perhaps further highlighted on the scree plot. However, given the large sample size (n=62), and the convergence of the scree plot and Kaiser's criterion on the two main components, these were retained in the final analysis.

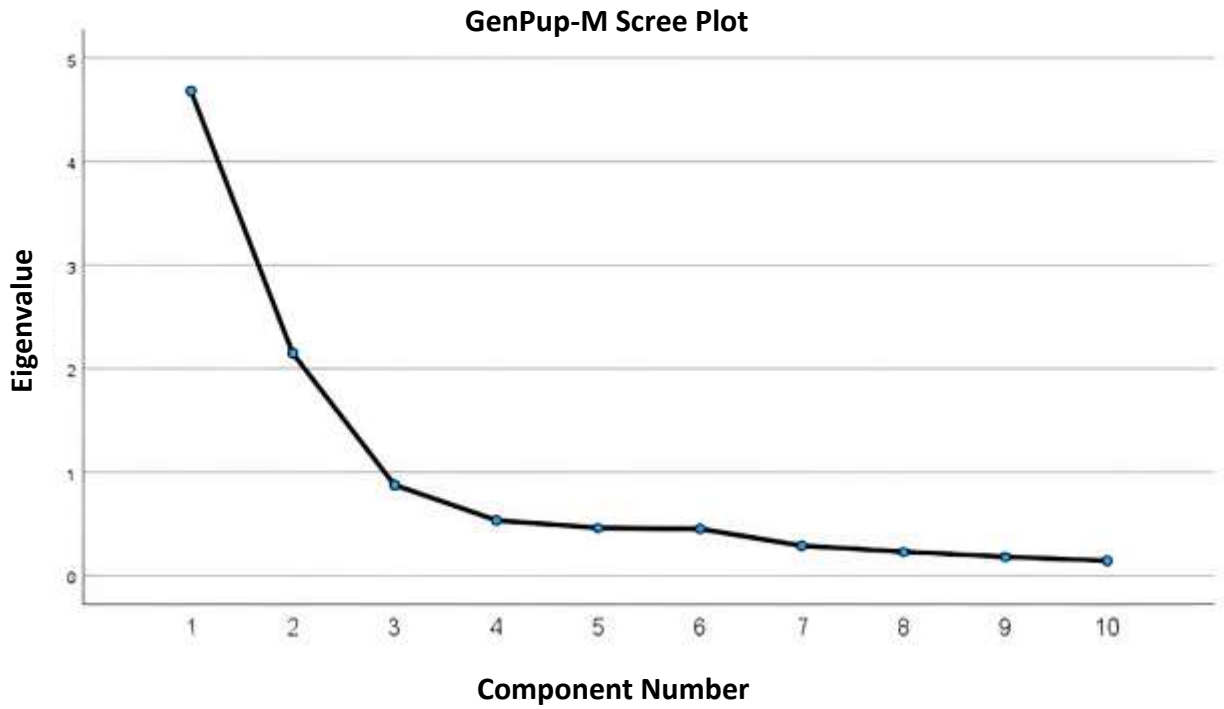

Figure 2. 3. Scree plot of the principal component analysis (PCA) of GenPup-M. There are two factors with Eigenvalues >1 (Component 1 and 2).

	INITIAL EIGENVALUES			EXTRACTION SUMS OF SQUARED LOADINGS			ROTATION SUMS OF SQUARED LOADINGS		
COMPONENT	**Total**	**Percentage of Variance**	**Cumulative Percentage**	**Total**	**Percentage of Variance**	**Cumulative Percentage**	**Total**	**Percentage of Variance**	**Cumulative Percentage**
1	4.682	46.815	46.815	4.682	46.815	46.815	4.367	43.674	43.674
2	2.149	21.488	68.303	2.149	21.488	68.303	2.463	24.630	68.303
3	0.876	8.761	77.064						
4	0.534	5.344	82.408						
5	0.461	4.611	87.019						
6	0.452	4.532	9.542						
7	0.289	2.885	94.429						
8	0.232	2.315	96.743						
9	0.182	1.821	98.564						
10	0.144	1.436	100.000						

Table 2. 7. Ten individual components from the GenPup-M questionnaire are showed with their associated factors. Components with Eigenvalues >1 were included in further principal component analysis (PCA). GenPup-M had two components which accounted for 68.3% cumulative percentage. There was scope to reduce the Eigenvalue limit to >0.5 to include a total of three components. However, Eigenvalues of >1 were chosen based on previous literature (Walton et al., 2013).

Table 2.2 below shows the component matrix of the factor loadings after varimax rotation. The items that cluster on the same components suggest that component 1 represents the effect of "stiffness/ease of movement" and component 2 the "willingness to be active/exercise"

	COMPONENT	
QUESTIONNAIRE ITEM	1	2
To what extent does stiffness reduce your dog's ability to exercise?	0.913	-0.193
How disabled is your dog by lameness?	0.890	-0.224
How much do you think your dog's mobility adversely influences his/her general well-being?	0.852	0.087
To what extent is your dog's mobility adversely affected immediately after exercise?	0.812	-0.071
To what degree does your dog show stiffness after a "lie down"?	0.774	0.023
To what extend does cold, damp weather reduce your dog's ability to exercise?	0.695	-0.405
How keen is your dog to exercise?	-0.058	0.890
How would you rate your dog's ability to exercise?	-0.231	0.809
How active is your dog?	-0.002	0.796
How often does your dog rest (stop/sit down) during exercise?	0.030	0.601

Table 2. 8. A varimax rotated component matrix showing two components. Factors heavily weigh on component 1, suggesting it is associated with "stiffness/ease of movement". Component 2 has factors heavily weighing on questions relating to activity, thus, component 2 was considered to represent "willingness to be active/exercise". Factor loading for the individual components are highlighted via the blue boxes.

Table 2.2 illustrates item loading for components extracted by PCA for analysis of GenPup-M, based on varimax rotations. Factor loading shows the correlation between the item and factor, typically, loading values of >0.4 shows good correlation between the item and the factor (Costello and Osborne, 2005). Communality signifies the proportion of variability between the item that is explained by the factor. A value for communality <0.4 can mean the item is not related to the others within that factor (Costello and Osborne, 2005).

QUESTIONNAIRE ITEM	Initial	Extraction
How much do you think your dog's mobility adversely influences his/her general well-being?	1.000	0.779
How disabled is your dog by lameness?	1.000	0.749
How active is your dog?	1.000	0.626
To what degree does your dog show stiffness after a "lie down"?	1.000	0.813
How keen is your dog to exercise?	1.000	0.773
How would you rate your dog's ability to exercise?	1.000	0.719
To what extent is your dog's mobility adversely affected immediately after exercise?	1.000	0.792
How often does your dog rest (stop/sit down) during exercise?	1.000	0.234
To what extend does cold, damp weather reduce your dog's ability to exercise?	1.000	0.629
To what extend does stiffness reduce your dog's ability to exercise?	1.000	0.671

Table 2. 9. A table to show each individual GenPup-M questionnaire item and their extraction factor weighting. The data shows good correlation between the questionnaire items which detect mobility impairments and extraction factors, as all questionnaire items relating to mobility have extraction loadings >0.4 suggesting the item is very well related to the factor.

2.3.3 *Cronbach's Alpha | GenPup-M*

The Cronbach's Alpha for the GenPup-M questionnaire containing 10 items was 0.87 overall (indicating "good" internal consistency, according to Tavakol and Dennick, 2011). The mean was 9.87, variance was 113.056, standard deviation was 10.6. Subscales were not used due to the nature of the questionnaire and based on previous literature (Walton et al., 2013; Hercock et al., 2009).

2.3.4 *Mann-Whitney U | GenPup-M*

A Mann-Whitney *U* test was used to see if there were differences in questionnaire responses for mobility impaired and non-mobility impaired dogs (as categorised according to clinical examination data). For both data sets, the responses were non-normally distributed. Total scores for GenPup-M were significantly higher for mobility impaired dogs compared to non-mobility impaired dogs (*U* value = 307.5, Z = -2.895, p-value = 0.004).

Standard error of skewness did not differ dramatically (0.403 for mobility impaired and 0.421 for non-mobility impaired). By examining the skewness of the data, it was confirmed that the scores for both mobility impaired and non-mobility impaired dog groups were significantly not normally distributed and significantly negatively skewed. Therefore, non-parametric statistical tests were used.

2.3.5 *Principal Component Analysis | LOAD*

Section 2.3.5 examines the LOAD questionnaire responses. The LOAD questionnaire was included in this study to assess criterion validity for GenPup-M. Identical statistical tests were used for both LOAD and GenPup-M to ensure a direct comparison was made. Previous PCA for the LOAD questionnaire were not used to ensure there were no differences in methodology or questionnaire responses.

A principal component analysis (PCA) was conducted on the 12 items with orthogonal rotation (varimax). The Kaiser–Meyer–Olkin (KMO) measure verified the sampling adequacy for the analysis, KMO = 0.81. All KMO values for individual items were > 0.77, which is above the acceptable limit of 0.5. Bartlett's test of sphericity χ^2 (45) = 459.891, $p < 0.001$, suggested that correlations between items were sufficiently large and PCA was favourable (Field, 2010).

Initial analysis was run to obtain Eigenvalues for each component in the data. Three components had Eigenvalues over Kaiser's criterion of 1 (Table 2.4). Percentage variance of component 1 (47.98%), component 2 (15.4%) and component 3 (9.32%) created a cumulative percentage variation amounting to 72.76%.

	INITIAL EIGENVALUES			EXTRACTION SUMS OF SQUARED LOADINGS			ROTATION SUMS OF SQUARED LOADINGS		
COMPONENT	**Total**	**Percentage of Variance**	**Cumulative Percentage**	**Total**	**Percentage of Variance**	**Cumulative Percentage**	**Total**	**Percentage of Variance**	**Cumulative Percentage**
1	5.758	47.987	47.987	5.758	47.987	47.987	4.687	39.061	39.061
2	1.854	15.452	63.439	1.854	15.452	63.439	2.907	24.222	63.283
3	1.118	9.318	72.757	1.118	9.318	72.757	1.137	9.474	72.757
4	0.698	5.813	78.570						
5	0.622	5.186	83.757						
6	0.476	3.967	87.724						
7	0.423	3.522	91.246						
8	0.339	2.824	94.070						
9	0.252	2.102	96.172						
10	.0.199	1.657	97.829						
11	0.166	1.384	99.213						
12	0.094	0.787	100.000						

Table 2. 10. Twelve individual components for Liverpool Osteoarthritis in Dogs (LOAD) questionnaire's ability in detecting mobility impairments are showed with their associated factors. Components with Eigenvalues >1 were included in further principal component analysis (PCA). LOAD had 3 components which accounted for 72.8% cumulative percentage. Three components were also extracted a previous study validating the LOAD questionnaire (Walton et al., 2013).

The scree plot (Figure 2.2) shows three components with Eigenvalues >1. Twelve components were identified, this is one component less than identified in the Walton et al (2013) study, where thirteen components were recognised.

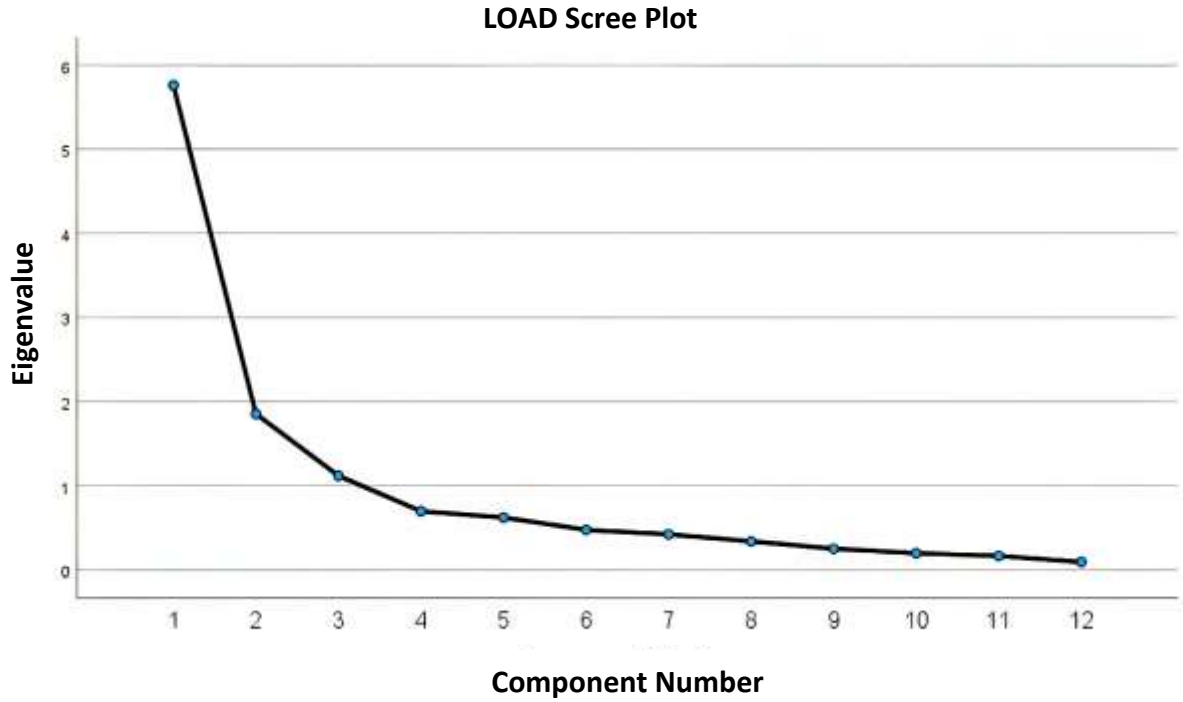

Figure 2. 4. Scree plot of the principal component analysis (PCA) of Liverpool Osetoarthritis in Dogs (LOAD). Three factors with Eigenvalues >1 are shown to be component number 1, 2 and 3.

Table 2.5 shows the component matrix of the factor loadings after varimax rotation. The items that cluster on the same components suggest that component 1 represents the effect of "stiffness/lameness", component 2 the "activity/exercise" and component 3 "attitude/demeanour". The previous work by Walton et al., 2013 also found three components for the LOAD questionnaire.

	Component		
QUESTIONNAIRE ITEM	1	2	3
To what extent does lameness impact your dog's ability to exercise?	0.896	-0.137	-0.038
How stiff is your dog after a lie down?	0.859	-0.094	0.083
To what extend does cold, damp weather impact your dog's lameness?	0.829	-0.238	-0.055
To what extent is your dog's lameness impacted after exercise?	0.806	-0.158	0.035
How disabled is your dog by lameness?	0.777	-0.127	0.095
To what degree does your dog show stiffness after a "lie down"?	0.765	-0.255	0.022
To what extend does cold, damp weather reduce your dog's ability to exercise?	-0.335	0.618	0.069
How keen is your dog to exercise?	-0.019	0.869	0.082
How often does your dog rest (stop/sit down) during exercise?	-0.018	0.858	-0.135
How would you rate your dog's ability to exercise?	-0.035	0.827	-0.305
How active is your dog?	0.154	0.645	0.390
How is your dog in him/herself?	0.116	-0.110	0.916

Table 2. 11. A table to show a varimax rotated component matrix showing three components for detecting mobility impairments within the Liverpool Osteoarthritis in Dogs (LAOD) questionnaire. Factors heavily weigh on component 1, suggesting it is associated with "stiffness/mobility impairment". Component 2 has factors heavily weighing on questions relating to activity, thus, component 2 was considered to represent "activity/exercise" and component 3 signifies "attitude/demeanour". Factor loading for the individual components are highlighted via the blue boxes.

Table 2.6 demonstrates item loading for components extracted by PCA for analysis of LOAD, based on varimax rotations. Each item above had a primary factor loading greater than 0.4, indicating good correlation between the item and the factor (Costello and Osborne, 2005).

QUESTIONNAIRE ITEM	Initial	Extraction
To what extent does lameness impact your dog's ability to exercise?	1.000	0.651
How stiff is your dog after a lie down?	1.000	0.747
To what extend does cold, damp weather impact your dog's lameness?	1.000	0.628
To what extent is your dog's lameness impacted after exercise?	1.000	0.781
How disabled is your dog by lameness?	1.000	0.800
To what degree does your dog show stiffness after a "lie down"?	1.000	0.694
To what extend does cold, damp weather reduce your dog's ability to exercise?	1.000	0.676
How keen is your dog to exercise?	1.000	0.500
How often does your dog rest (stop/sit down) during exercise?	1.000	0.753
How would you rate your dog's ability to exercise?	1.000	0.823
How active is your dog?	1.000	0.812
How is your dog in him/herself?	1.000	0.866

Table 2. 12. A table to show each individual Liverpool Osteoarthritis in Dogs (LOAD) questionnaire item and their extraction factor weighting. There was a good correlation between the above items and extraction factor loading, as all factor loadings >0.4 suggesting the questionnaire item is related to the factor.

2.3.6 *Cronbach's Alpha | LOAD*

The Cronbach's Alpha for the LOAD questionnaire containing 12 items was 0.92 overall (indicating "excellent" internal consistency, according to Tavakol and Dennick, 2011). The mean overall score was 5.23, variance was 68.68, standard deviation was 8.29. Subscales were not used due to the nature of the questionnaire and based on previous literature (Walton et al., 2013; Hercock et al., 2009).

2.3.7 *Mann-Whitney U | LOAD*

A Mann-Whitney *U* statistical test was used to see if there were differences in questionnaire responses for mobility impaired and non-mobility impaired dogs. The mean overall LOAD scores for owners with mobility impaired dogs were only slightly higher than those with non-mobility impaired dogs (46.8, compared to 44.6, respectively), the standard deviation for questionnaire responses regarding mobility impaired dogs was 9.46 compared to 6.4 for non-mobility impaired dogs. For both data sets, the responses were non-normally distributed. Differences in LOAD scores between mobility impaired and non-mobility impaired dogs include: *U* value = 433.5, Z value = -1.059, p-value = 0.029. The mean ranks were not equal (34.86 for mobility impaired, 29.98 for non-mobility impaired).

Similarly, to the GenPup-M questionnaire results, the standard error of skewness for the LOAD questionnaire did not have meaningful differences (.409 for mobility impaired and .421 for non-mobility impaired). By analysing the "degree of skewness", it was concluded the scores for mobility impaired and non-mobility impaired dogs were significantly not normally distributed and significantly negatively skewed. Therefore, non-parametric statistical tests were used in the analysis of this data.

2.3.8 *Receiver Operating Characteristic (ROC) Curve*

To obtain summative statistical measures of GenPup-M's diagnostic ability to determine mobility impaired dogs from non-mobility impaired dogs, a Receiver Operating Characteristic (ROC) curve was used. GenPup-M was subjected to the area under the curve (AUC) analysis of ROC curves. A 95% confidence interval was used to establish binary logistic regression with a significance level of 5%. The ROC analysis results were interpreted as follows: area under the curve (AUC) <0.70, low diagnostic accuracy; AUC in the range of 0.70–0.90, moderate diagnostic accuracy; and AUC ≥0.90, high diagnostic accuracy (Swets, 1996). Correspondence between the scales was already assessed with a Spearman correlation test. The AUC for GenPup-M was 0.81, with a sensitivity of 74.2%, which suggests reliance on GenPup-M would produce a small number of false positives (25.8%) when determining canine mobility impairments. False positives were determined as the percentage of dogs that did/did not

have mobility problems (as determined by the validated veterinary clinical examination) that were incorrectly identified as having/not having mobility problems.

2.4 Discussion

Principal component analysis (PCA) helps to identify the correlation between questionnaire items and highlights any dependencies among the data set. Walton et al (2013) used PCA to identify specific components which helped in the detection of canine mobility impairments. Using PCA for GenPup-M has helped identify questionnaire components which correlate to the detection of mobility impairments, but also help to determine the correct statistical tests to undertake when comparing GenPup-M to other methods of canine gait analysis.

Two main components were identified with Eigenvalues >1 during the Principal Component Analysis (PCA) for GenPup-M. The two components were categorised into: Component 1; "stiffness/ease of movement" and component 2; "willingness to be active/exercise". The Liverpool Osteoarthritis in Dogs (LOAD) mobility questionnaire has been previously validated (Walton et al., 2013) and was used to determine construct validity of GenPup-M. The LOAD questionnaire also underwent PCA and three components were extracted; component 1; "stiffness/lameness", component 2; "activity/exercise" and component 3; "attitude/demeanour". These components differed slightly from the original Walton et al paper, where component 3 was "effect of weather". However, since exact methodology for coding and extracting questionnaire data was not published by Walton et al, these minor differences could be accounted for by changes in coding and analysis strategies.

The GenPup-M questionnaire showed good internal consistency (0.87) when compared to previously validated mobility questionnaires: LOAD = 0.88, HCPI = 0.83, and CBPI = 0.92 (Walton et al., 2013). This indicates that the GenPup-M questionnaire is reliable and the items within the questionnaire are closely related to measuring mobility impairments. Total scores on the GenPup were significantly higher for mobility impaired dogs, compared to non-mobility impaired dogs. There was an average 10-point score difference between the mild and moderately mobility impaired dogs (mean (SEM) and SD was: 33.72 (2.03) ± 10.15 for mild, and 44 (2.58) ± 6.32 10.28 for moderate dogs) highlighting that GenPup-M questions

are useful and able to differentiate between levels of mobility impairments. However, it would be interesting to assess the score differences between mildly lame dogs and severely lame dogs.

The AUC value (0.81) is considered 'good' (Safari et al., 2016) and gave 25.8% probability of GenPup-M incorrectly detecting a mobility impairment. The ROC/AUC analysis is scarcely used in veterinary medicine when developing scoring systems, yet, is commonly used in human medicine. For example, A 2008 human orthopaedic study aimed to develop and validate a pre-operative scoring system to assess the chance of mortality in patients undergoing hip fracture surgery, the AUC analysis returned a score of 0.72 (acceptable, with a 34.5% chance of false-positive results) (Maxwell et al., 2008). This value was considered to be better than another human orthopaedic study which aimed to assess mortality 30 days after surgery for a fractured neck of the femur, where the ROC analysis returned an AUC value of 0.62 (poor, with a 44.5% chance of false-positive results). However, in any case, it is important to remember that a ROC curve only tries to predict the *probability* that an event will occur for any individual. In the present study, the AUC result may have been influenced due to many participants only having mild or mild-moderate mobility impairments, and thus, conflicting answers within this GenPup-M cohort could have accounted for the AUC result as some dogs intermittently showed mobility impairments. Although, GenPup-M is designed to be used as part of a suite of diagnostic measures, including clinical examination, therefore, the questionnaire is merely a way of quantifying owner reports and helping them identify changes in their dog's behaviour or mobility for discussion with their veterinary surgeon, GenPup-M is not intended to replace clinical examination, clinical history or diagnostic imaging for the diagnosis of mobility impairments.

This project aimed to validate GenPup-M for use in clinically healthy dogs, alongside those with mobility impairments (subtle or chronic) due to orthopaedic or neurological disease. Once validated, GenPup-M will be used to provide longitudinal assessment of canine mobility impairments within a veterinary setting. For this study, GenPup-M will be validated by comparing owner-reported answers to a previously validated canine mobility CMI (LOAD questionnaire), a validated clinical examination sheet (Harris et al., 2018), and 3D gait analysis. For this project, the validated clinical examination will be used as the gold standard outcome measure for canine mobility impairments and be used to categorise dogs as either

mobility impaired or non-mobility impaired. This methodology will be similar to previous research (Muller et al., 2016; Walton et al., 2013; Hercock et al., 2009; Hielm-Björkman et al., 2003) whereby clinical history and clinical examination data were obtained by an orthopaedic specialist to assess mobility status and determine the inclusion/exclusion of participants onto the study. Harris et al., 2018 also used their validated clinical examination sheet (the same sheet used in the current study) to assign dogs to the OA group for analysis, since radiographs were not obtained for the cohort.

2.5 Conclusion

GenPup-M was determined to have two constructs for detecting the presence of mobility impairments: “stiffness/ease of movement” and “willingness to be active/exercise”. There was a statistically significant difference between overall average owner-reported GenPup-M scores for mild and moderately mobility impaired dogs, this was further supported by GenPup-M having a ‘good’ Cronbach Alpha result which supported its internal consistency. The comparable results of LOAD, CBPI and HCPI from previous work provides further evaluation of GenPup-M’s reproducibility (agreement and reliability) and additional validation of the scale.

CHAPTER THREE | VETERINARY CLINICAL EXAMINATION

3.1 Background

The musculoskeletal system is comprised of an abundance of muscles, tendons, ligaments, bones and joints to aid ambulation and locomotion (Arnoczky and Tarvin, 1981). Dysfunction of this system can result in abnormal gait and mobility (Anderson et al., 2018). Neurological disorders can also result in non-ambulatory patients (Arnoczky and Tarvin, 1981).

As discussed in Sections 1.2 and 1.1.3, in order to accurately perform an orthopaedic examination, the veterinary surgeon must obtain a complete assessment of history, gait, conformation and physical status of the animal (Anderson et al., 2018). The examination must also be thorough and organised by following a methodical order (Arnoczky and Tarvin, 1981).

Veterinary orthopaedic clinical examinations have also been used in conjunction with kinetic or kinematic gait analysis as discussed in Section 1.2 (Walton et al., 2013; Hercock et al., 2009; Millis and Mankin., 2014; Waxman, 2008). Although orthopaedic clinical examination is not considered to be superior to gait analysis, its use should not be undermined in clinical practice where kinetic or kinematic analysis is not always accessible (Waxman et al. 2005).

3.2 Materials and Methods

3.2.1 *Collection of Veterinary Clinical Examination Data*

The previously validated veterinary clinical examination sheet (Harris et al., 2018) (Supplementary Material 4 and 5) was completed for each dog. The veterinary clinical examination was performed by a qualified veterinary surgeon (EC or NC). Clinical examination was undertaken prior to 3D gait analysis and consisted of both a visual gait assessment and manual mobilisation of all four limbs to identify any mobility problems (Harris et al., 2018). All of the examinations followed the same methodical structure and were carried out in the gait laboratory at the WHD building. All dogs were allowed to acclimatise to the room for 10

minutes before testing began. Dogs were minimally restrained throughout the procedure to allow natural behavioural responses to discomfort (Walton et al., 2013).

Each clinical examination sheet was in paper format and the date, canine participant ID number, clinical name and dog name were recorded in the relevant boxes. Dog age, breed, sex and neuter status were also noted for further analysis. Weight in kilograms (kg) and body condition score (BCS) was entered using the 1-9 scale adapted by Laflamme (1997) (Figure 2.1): thin (1-3), ideal weight (4-5) and overweight (6-9). Additionally, dogs were categorised into sizes according to the breed standards of the UK Kennel Club: small (<10Kg), medium (10-25Kg), large (25-45Kg) and extra-large (>45Kg).

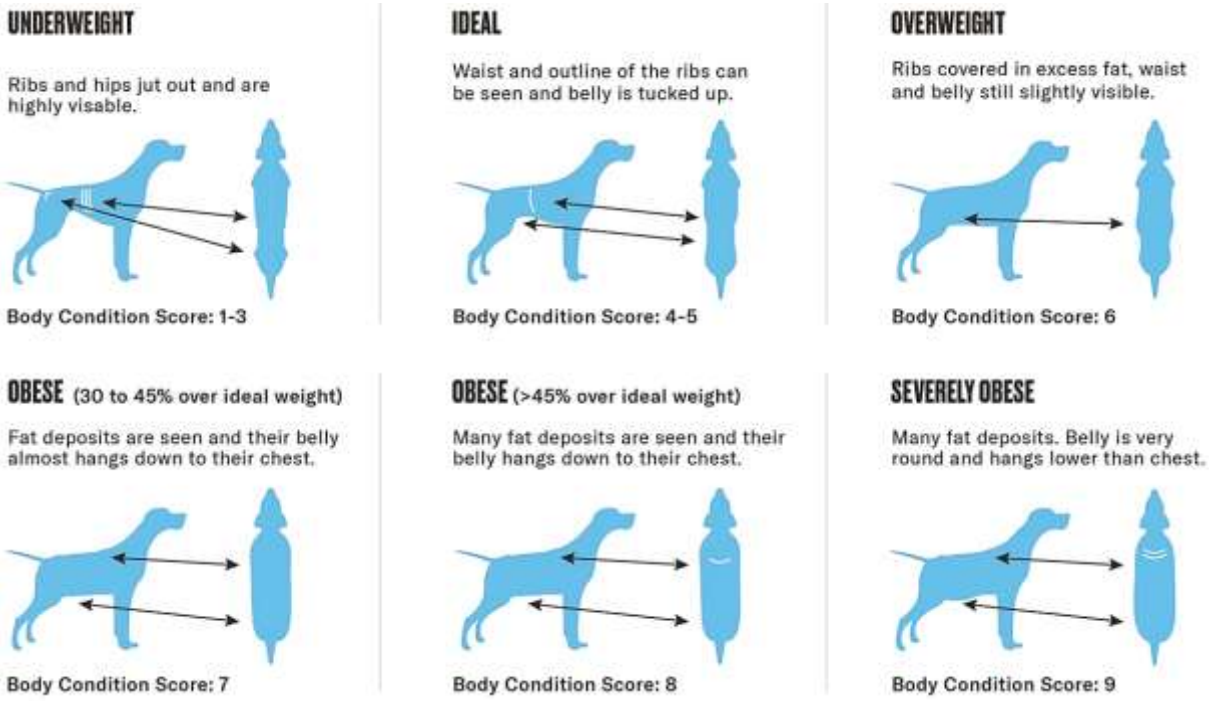

Figure 3. 2. Canine body condition score (BCS) guidelines adapted from Chun et al (2019). BCS 1-3 are classed as 'underweight', BCS 4-5 are 'ideal' and BCS of >7 are 'obese' and 'severely obese'. veterinary surgeon

Using the previously validated clinical examination sheet by Harris et al (2018), dog owners were asked to provide a summary of their dog's orthopaedic problems (if applicable), along with any other health-related issues. Mobility was determined by assessing lameness during locomotion (Vasseur, 1993) using a numeric rating scale (NRS) of 0-10, where '0' represents a

“sound” limb (e.g. “no pain”) and '10' represents a dog who ‘places the toe when standing, but carries limb when trotting’ was used to determine an overall lameness score and identifying if any limbs were affected. A numeric rating scale (0-3) was also used to score the “stand-up/lie-down”, where ‘0’ indicated normal function, and ‘3’ highlighted great difficulty when manoeuvring from a sitting to a lying position (or vice versa). Thoracic and pelvic limbs were assessed individually, whereby digit joints, carpi, elbows, shoulders, tarsi, stifles and hips were graded (0-4) using a Joint Function Score (JFS) adapted by Impellizeri et al., (2000), where ‘0’ meant a normal range of motion (ROM) and ‘4’ meant three-four joints were affected in a single limb and a pain response was elicited upon touch of a joint. The JFS allowed the identification of joint abnormalities, crepitus, tissue swelling, effusion, abnormal bone structure, muscle atrophy. These impairments were translated to give an overall global score of ‘none’, ‘mild’, ‘moderate’ and ‘severe’ for the severity of the dog’s condition (Harris et al., 2018). All dogs underwent a brief neurological assessment as explained in Supplementary materials 4, 5 and 6; cranial nerves, menace and tracking reflexes were assessed during the head and neck component of the examination, knuckling reflexes were assessed for all 4 limbs and anal tone was occasionally evaluated in association with the ‘tail jack’. A more thorough neurological examination was completed on 3 dogs who presented with ataxia due to a previous fibrocartilaginous embolism (FCE) diagnosis, this comprehensive neurological component investigated posture (e.g. kyphosis, lordosis, head tilts) and gait (head and neck turns, wide-based stance, etc), pain on spinal palpation, pain perception and postural reaction assessments (e.g. paw replacement, wheelbarrowing and hopping exercises) to identify proprioceptive deficits. This above neurological examination component was adapted from (Paluš, 2014 and Stogdale, 2002).

3.2.2 *Analysis of Veterinary Clinical Examination Data*

All veterinary clinical examinations were completed by a qualified veterinary surgeon (NC/EC), with initial training by an orthopaedic specialist (EC). Therefore, the researchers were not blinded as to the mobility status of the dog. As per Harris et al (2018), clinical examination paper responses were recorded by the researcher and then transferred into an MS Excel (Office 2019) document for further coding.

Overall veterinary clinical examination scores and PVF values were compared to GenPup-M scores to support construct validity. Spearman's rank correlation was used to compare responses ($p \leq 0.05$, two-tailed) and differences between clinical examination scores within mobility impaired and non-mobility impaired groups were compared via a Mann-Whitney U test.

3.3 Results

3.3.1 *Canine Participant Signalment*

Seventy-one dogs were recruited onto the project initially, however, three dogs were discarded from this study, due to not fulfilling the inclusion criteria due to being placed on pain relief medication prior to investigation. A further six dogs (four Chihuahuas and two Shih-Tzus) were discarded from the gait analysis due having a body weight <4kg and thus, inconclusive kinetic data, in addition, there was insufficient visualisation of the retro-reflective markers for kinematic data analysis. Therefore, there total number of dogs that were included in the data analysis for this study was sixty-two. Table 3.1 below provides demographic details (including sex, age, bodyweight, breed and joint affected) of the sixty-two dogs involved in this study.

Standard Error of the Mean (SEM) is shown in Table 3.1 alongside age and weight variables. A "combination" classification was created based on previous literature (Walton et al., 2013). Dogs were assigned to this category if they had multiple limbs affected and the researcher was unable to identify which limb was most severely affected. Dogs were categorised as mobility impaired based on the owner-reported clinical history and veterinary clinical examination data undertaken prior to gait analysis.

Variable		
Gender (number)	Entire Male (%)	3 (4.8%)
	Neutered Male (%)	22 (35.5%)
	Entire Female (%)	8 (12.9%)
	Neutered Female (%)	29 (46.8%)
Age (Months)	Mean (SEM)	72.8 (5.26)
	Minimum	5
	Maximum	192
Bodyweight (kg)	Mean (SEM)	21.4 (1.24)
	Minimum	6.4
	Maximum	37.2
Breed (number)	Number of breeds represented	17
	Cross Breed (%)	13 (20.9%)
	Labrador Retriever (%)	12 (19.4%)
	Small Terrier breeds (%)	8 (12.9%)
	English Springer Spaniel (%)	6 (9.7%)
	Border Collie (%)	3 (4.8%)
	Other (%)	20 (32.3%)
Joint Affected (number)	Elbow (%)	10 (16.1%)
	Stifle (%)	6 (9.7%)
	Hip (%)	6 (9.7%)
	Carpus (%)	2 (3.2%)
	Tarsus (%)	0 (0%)
	Shoulder (%)	0 (0%)
	Combination (%)	7 (11.3%)
	None affected (%)	31 (50%)

Table 3. 3. Demographic data of the UoL Mobility cohort (n=62) cohort. Gender, age, body weight, breed and joint affected is shown. Percentages for each category is also represented for the data set. The joint affected is included for the mobility and non-mobility impaired cohorts.

Of the n=62 dogs that were assessed by clinical examination, 31 were assessed to have a mobility impairment. Of the 31 with mobility impairments, only 13 of these dogs' owners reported a history of mobility impairment. The highest overall combined clinical examination score from the above categories could be twenty-six (including BCS) and seventeen (excluding BCS). Table 3.2 provides an overview of the frequency each score occurred for mobility impaired and non-mobility impaired dogs excluding BCS values.

Mobility Impaired	Overall Clinical Examination Score	Frequency	Percentage	Cumulative Percentage
No	0.00	31	100.0	100.0
Yes	3.00	4	12.9	12.9
	4.00	4	12.9	25.8
	5.00	3	9.7	35.5
	6.00	10	32.3	67.7
	7.00	3	3.7	77.4
	8.00	2	6.5	83.9
	9.00	3	9.7	93.5
	13.00	1	3.2	96.8
	14.00	1	3.2	100.0
	Total	31	100.0	

Table 3. 4. A table to show a summary of the frequency at which overall scores occurred for dogs with and without mobility impairments (excluding the BCS score values). The lowest score of four mobility impaired dogs was 4/17 (12.9%), and the highest score given for one dog was 14/17 (3.2%). All non-mobility impaired dogs had a clinical examination score of 0.00. The clinical examination scoring system was used to categorise mobility impaired dogs and non-mobility impaired dogs, alongside the severity of the mobility impairments.

3.3.2 *Data Analysis*

The variance between overall combined clinical examination scores for mobility impaired dogs was 6.8 and the standard error of skewness was 0.42. The skewness value in this data set for mobility impaired dogs was 1.31, showing the data were non-normally distributed and significantly positively skewed with the standard deviation being 2.61.

A Mann-Whitney *U* statistical test was therefore used to see if there was a statistical difference in overall clinical examination scores between mobility impaired and non-mobility impaired dogs. Mobility impaired dogs had significantly higher clinical examination scores than non-mobility impaired dogs (*U* value = 496.0, Z = -7.252 and p = <0.001). The mean ranks were not equal (47.0 for Mobility impaired, 16.0 for non-mobility impaired).

The effects of age, body condition score (BCS), and breed on the degree of mobility impairment were investigated by using data from the validated clinical examination sheet. Age showed a positive correlation with clinical examination scores (0.663, P<0.001), In Figure 3.2, age significantly correlated (p<0.01, two-tailed) with overall lameness score, stand-up/sit-down, the specific joint abnormality and joint function score (JFS) (0.631, 0.583, 0.609, and 0.695, respectively).

A chi-square test of independence was performed on body condition score (BCS) to examine the relationship between BCS and overall clinical examination score. The relationship between these variables was not significant, X^2 (36, N = 62) = 23.652, p = 0.943. Thus, BCS had no effect on the overall degree of mobility impairment in this study.

3.4 Discussion

A validated veterinary mobility clinical examination sheet (Harris et al., 2018) was used in this study to categorise dogs into mobility impaired and non-mobility impaired categories, and to obtain data relating to five categories; body condition score (BCS) (1-9), overall mobility impaired score (0-9), Stand-up/sit-down score (0-3), joint abnormality score (0-1) and joint function score (0-4). A full clinical history was also taken from the owners prior to examination. Furthermore, dogs in this study could only be recruited if they fit the inclusion criteria of not receiving any pain relief medications which could mask mobility impairments. Furthermore, the requirement that all dogs recruited would be required to walk a total of 175 metres (5 metres x 35 trials) during the gait analysis session made some owners decide not to participate in this investigation, thus, the dogs could not undergo a veterinary clinical examination.

Of the 62 dogs that were assessed by clinical examination, 31 were assessed to have a mobility impairment. Of the 31 with mobility impairments, only 13 of these dogs' owners reported a history of mobility impairment. Nineteen dogs were categorised as having either mild mobility impairments, and ten dogs were categoriesed as having moderate mobility impairments. No dogs were identified to have severe mobility impairments on clinical examination by physical or visual gait assessment. Waxman et al (2006) suggested that, except for severe lameness,

visual gait assessment was largely considered inaccurate. In this study, the identification of mobility impairments using clinical examination was supported by kinetic and kinematic gait analysis (Section 3.2). Nonetheless, it is important to note that although gait analysis and clinical examination findings may overlap, they are not equivalent and may provide different information surrounding mobility impairments. For example, quantitative gait evaluation can objectively assess joint motion and stride characteristics to provide information relating to acceleration, swing time, stance time and stride length which can be useful when determining lameness (Millis and Janas, 2021). However, there are several factors which can cause skewed data and anomalous results, e.g. having an inexperienced handler who walks the dogs at inconsistent speeds during the trial alter overall results. McLaughlin and Roush, 1994 found that increased velocity can decrease stance time, in turn, making it look like the animal wanted to continually shift its weight from limb to limb, and possibly be misinterpreted as having a mobility impairment. Although once potential variables have been minimised, kinetic and kinematic gait analysis can provide an objective, quantifiable and repeatable information on normal and abnormal gait in dogs, and can prove very useful when comparing data from the same population at different time points (i.e. before and after treatment), where in contrast, a dog may be clinically examined by a different veterinary surgeon at both time points, thus, increasing to subjectivity for improvement (Waxman et al., 2008). Further explanation for the use of clinical examination and gait analysis in the detection of orthopaedic conditions causing mobility impairments can be found in Chapter 4 (Section 4.4.4)

It is important to note that clinical examination and qualitative gait analysis both occur in control, clinical environments, which may cause animals to hide subtle changes in their gait or become more stoic, hence, clinical history must not be disregarded to gait a comprehensive view of the animal's mobility in a natural environment. A recent human study (Guinet, Néjib and Eric, 2020) found that clinical gait analysis and clinical examination did not correlate with the actual daily physical activity levels (using accelerometers) of children with cerebral palsy, the study concluded that gait analysis and clinical examination could only partially reflect the overall level and quantity of daily physical activity children living with cerebral palsy could endure and that neither gait analysis nor clinical examination could strongly determine physical activity. Although no veterinary study has investigated the relationship between gait

analysis and clinical examination in determining activity levels for dogs with orthopaedic or neurological conditions, it would be prudent to solely base diagnosis on one technique alone as each will provide differing information. Similarly, good clinical history or clinical metrology instruments (CMIs) should be used to provide information relating to activity levels and ease of movement outside of the clinical environment.

In this study, the validated clinical examination (Harris et al., 2018) was able to detect differences between mobility impaired and non-mobility impaired dogs 3.3. Mobility impaired dogs had significantly higher clinical examination scores than non-mobility impaired dogs (*U* value = 496.0, Z = -7.252 and p = <0.001). This is unsurprising as the clinical examination sheet is based on an orthopaedic veterinary assessment, which is what was used to categorise dogs into mobility impaired and non-mobility impaired. However, future studies could investigate the impact of comparing GenPup-M scores with a subgroup of mobility impaired dogs with relatively low clinical examination scores (for example, scores 3-6) to see if there are any meaningful statistical differences observed. Unsurprisingly, there was a positive correlation between age and overall clinical examination score (r^2 = 0.66, p<0.001) with increasing severity of mobility impairments. Body condition score and breed were found to have no significant relationship with increasing overall clinical examination scores.

3.5 Conclusion

There were statistically significant differences between mobility impaired and non-mobility impaired dogs in our study when interpreting veterinary clinical examination scores. Age was found to have an impact on clinical examination scores, with older dogs scoring higher values compared to younger dogs, supporting the assumption that older dogs have higher incidences of mobility impairments, or perhaps, is a coincidence due to a small sample size. Thus, more work would need to be completed on the association with clinical examination mobility scores against the increasing age of dogs. Similarly, there were no relationships between breed or BCS in this cohort, again, perhaps, this was due to the wide demographic of dogs within a small population, as this assumption does not agree with previous studies (Anderson et al., 2018; Rychel, 2009).

CHAPTER FOUR | CANINE KINETIC AND KINEMATIC GAIT ANALYSIS

4.1 Background

Many objective systems are available to assist veterinary surgeons in the detection of canine mobility impairments (Kim, 2008). Simply, camera or smartphone systems can be used to video-record a dog's gait for assessment of dysfunction (Zink et al, 2013). However, more complex systems are available, as discussed in section 1.2.1, that can be used to record information relating to stance and swing phases, ground reaction forces (GRFs) and velocity and uneven weight distribution (Cook, 2001) as discussed in Section 1.2.2.

Force plate analysis is the most common format of kinetic analysis (Section 1.2.2). It allows GRFs to be measured. From this, Peak Vertical Forces (PVF) and vertical impulse (VI) of a limb can be calculated. Kinetic gait analysis can only measure one side of the animal at a time, thus, may require multiple passes along the force plates to obtain consistent results. This acquisition of data can be detrimental if multiple passes would result in increased lameness and pain for the animal or if the animal was too mobility impaired to continually repeat the process. PVF and VI are thought to decrease as lameness increases, therefore, they are still the most commonly used objective indices to detect mobility impairments (Sanderson et al., 2019).

Kinematic gait analysis (section 1.2.3) commonly uses retro-reflective markers which are attached to the animal's skin at various anatomical locations. Data relating to velocity, joint angles, acceleration and deceleration can be extracted by tracking the motion of these markers. 2D and 3D models can then be created by transferring data captured by a series of motion capture cameras. ROM can also be obtained from retro-reflective markers (Sanderson et al., 2019).

Quantitative canine gait analysis will be used in the current project to enable further validation for GenPup-M, and to support veterinary clinical examination findings in the detection and categorisation of mobility impairments.

4.2 Materials and Methods

4.2.1. *Kinetic and Kinematic Canine Gait Data Collection*

4.2.1.1 *Set up and Calibration*

Time synchronised force plate recordings and 3D spatiotemporal kinematics were recorded for all dogs recruited in this study. Qualisys Oqus-7 motion cameras (Figure 4.3 (A)) recorded spatiotemporal kinematic data via retro-reflective markers. Time synchronised ground reaction forces (GFR) were collected Kistler force (Type 9260AA, Kistler UK) plates (Figure 4.3 (B)). Prior to analysing the dogs' gait, all twelve Qualisys Oqus-7 cameras were calibrated. The calibration frame was constructed with a metal 'L' shaped pole containing four retro-reflective markers. This calibration tool had to be placed along the x and y axis of one of the three floor-mounted force plates to be identified by video software and create a global coordinate system (GCS) (Sandberg et al., 2020).

A 'T' shaped metal calibration wand (also containing two retro-reflective markers of x, y and z coordinates) was moved in various directions for a minimum of two minutes to emulate a dog's movement pattern and determine the GCS relative to the recording space (Sandberg et al., 2020). Calibration was performed at the start of each data collection session and all twelve cameras remained turned on throughout the entire data collection period. The location of a succession of three Kistler forces plates, mounted within a floor recess, was also calibrated spatially as part of this process. The three orthogonal ground reaction forces mediolateral (Fx), craniocaudal (Fy) and vertical (Fz) measured by these plates were recorded synchronously with motion capture within the camera software, enabling quantifiable data to be obtained for the assessment and evaluation between kinematic variables of mobility impaired and non-mobility impaired dogs (Figure 4.1). Vertical (Fz) was used in this study to obtain Peak Vertical Force readings.

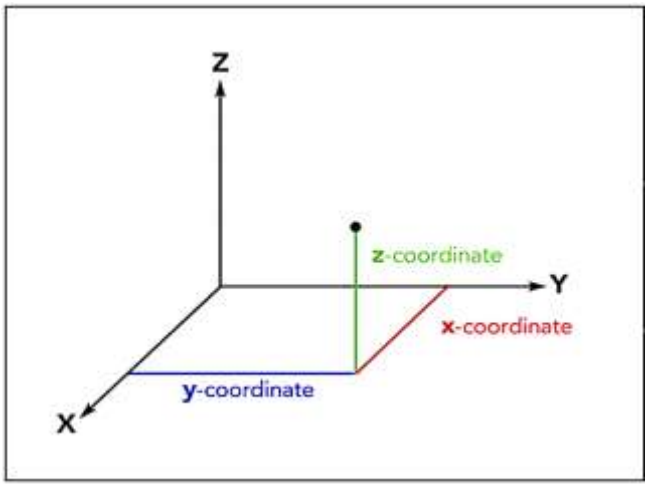

Figure 4. 5. An illustration of the forces measured during kinetic force plate gait analysis. Based on Carr and Dycus (2016). Forces in the Z direction are generated by vertical compression onto the force plate, the force generated along the Z axis is greater than those produced in the X or Y direction. Subsequently, peak vertical force (PVF) is calculated from forces exerted in the Z direction.

4.2.1.2 *Marker Placement and Gait Analysis Trials Acquisition*

Forty-four small spherical adhesive retro-reflective markers were placed at specific points on each dog (Figure 4.4 and Supplementary Material 8). Markers with a 12.7mm diameter (smaller, 9.7mm diameter markers were used in dogs under 13kg to aid accurate data recording) were placed symmetrically on the left and right side of the dog (distance between markers varied with the size and breed of dog). Markers on the foot were used to derive stride/step lengths and widths, and duty factors, whereas, markers on the torso aimed to derive gait velocity. The number of retro-reflective markers (44) was chosen based on a previous study by Sandberg et al (2019), whereby, this number will allow gait data to be obtained relating to 3D motion and translation of major limb segments, although analysis of body segment kinematics was beyond the scope of this study. The marker sites were chosen specifically for being in an area with minimal subcutaneous tissue, thus, reducing oscillations and measurements of unrelated movements being recorded. The placement of markers was always undertaken by the same operator (NC) due to previous studies highlighting the impact of incorrect marker position on canine gait analysis (Kim et al., 2017; Torres et al., 2011). In some cases, the fur of long-haired breed dogs covered the reflective markers. In these

instances, a small patch of fur was shaved to allow the markers to become visible to the cameras. The potential need to clip/shave fur was included in the information sheet, to which the owners gave informed consent.

Based on similar studies (Walton et al., 2013, Hercock et al., 2008); Kano et al., 2016), each dog was walked along the walkway and across the force plates by the same handler during the trial. A maximum of three handlers (excluding dog owners who were allowed into the WHD building) were used throughout this study. Handlers were never switched mid-data collection. A non-reflective 2-metre lead was used for all dogs to ensure the handler did not actively interfere with the pace speed and allowed the dogs to move at a self-selected velocity (Colborne et al, 2011). The non-reflective quality of lead ensured the motion capture cameras did not mistake any lead reflectivity for retro-reflective markers. The walkway measured 10m x 0.5m and consisted of rubber flooring to ensure the surface was non-slip (Figure 4.2)

Figure 4. 6. Image of the William Henry Duncan Building gait laboratory with the start and end of the walkway over the force plates (illustrated with black arrows). Each dog was trotted along this walkway containing three Kistler force plates a maximum of 25 times, and for a further 10 walking trials if deemed acceptable and would not hinder the dog's welfare.

In all cases, a maximum of twenty-five trotting trials per dog were collected, where the dog moved continuously along a straight-line walkway with floor-mounted force plates. Trotting gait was used as this is the most common gait analysed in veterinary mobility literature (Carr et al., 2015) and a natural speed for many dogs. Walking and trotting trials were only deemed successful when all four feet has clean strikes with two out of three force plates during the trial (Kano et al., 2016; Walton et al., 2013), and when dogs were observed to traverse the walkway continuously, in a straight line, without distractions.

Clean water bowls were provided for each dog while they were present in the WHD building, Data collection (including a fully veterinary clinical assessment and gait analysis) required approximately 1.5-2 hours to complete.

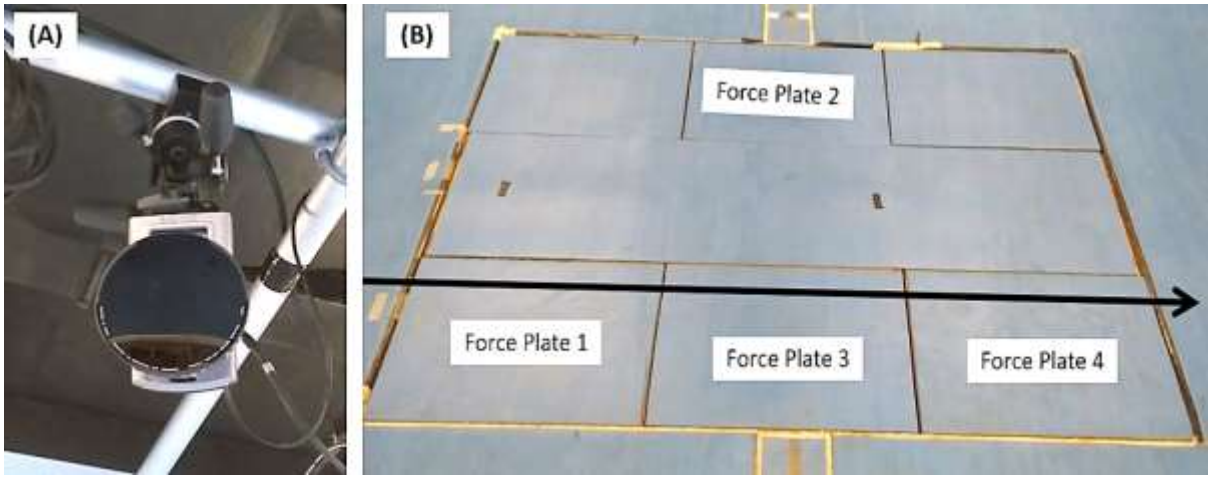

Figure 4. 7. (A) One Qualisys Oqus-7 motion cameras suspended from scaffolding (12 in total were used in this study). (B) Kistler force plates (Type 9260AA, Kistler UK). Force plates 1, 3 and 4 were used in this study. Dogs were trotted across the force plates in the direction of the black arrow.

All dogs were assigned as mobility impaired or non-mobility impaired prior to kinetic or kinematic gait analysis being completed. The categorisation was determined by using a validated clinical examination sheet (Harris et al., 2018) and used similar methodology from similar previous veterinary mobility studies (Hercock et al., 2009; Hielm-Björkman et al., 2003; Muller et al., 2016; Walton et al., 2013), Harris et al., 2018 also used their validated clinical examination sheet (the same sheet used in the current study) to assign dogs into the OA group for analysis, since radiographs were not obtained for the cohort.

4.2.2 *Kinetic and Kinematic Canine Gait Data Analysis*

Qualisys Track Manager (QTM) (version 2.15, build 3300) was predominantly used to process and clean kinematic data prior to statistical analysis in MATLAB (MATLAB, 2020. version 9.9.0.1592791 Update 5 (R2020b).

All 25 trotting trials and 10 walking trials were placed into individual folders with the corresponding participant ID number for cleaning. Following this, manual individual labelling of all forty-four retro-reflective markers with the correct anatomical location was required.

As reported by Pascal (2003), manual labelling is required to ensure the markers are identified correctly, the computer system did not process the markers in the wrong anatomical location. This was a time-consuming task which required the utmost precision. To decrease the time spent manually labelling markers, a model could be created and applied. The pre-defined model could be created by manually labelling one full trial, exporting the named marker positions as a 'stick' model, and then applying said model to subsequent trials for that same dog. This technique never correctly identified 100% of the unlabelled markers but did allow 50-75% of markers to be correctly identified automatically, thus, leaving a small proportion of markers to be manually labelled.

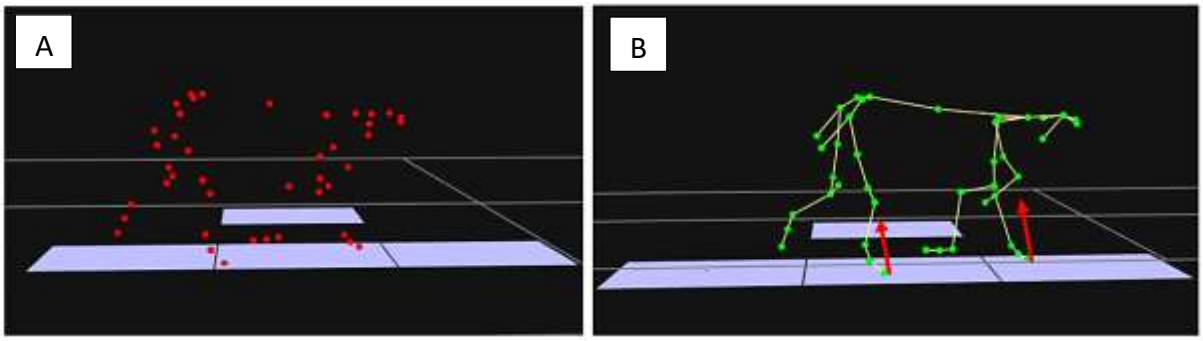

Figure 4. 8. A still image from an animation to represent 44 unlabelled retro-reflective markers at various anatomical locations including: frontal lobes, occipital crest, C7, spine of the scapula, T13, L7, wings of the ilium, acromion, mid-humerus and mid-femur, lateral epicondyle of the humerus and femur, ulnar styloid, femorotibial joint, malleolus, carpus, tarsus, metocarpo-/tarso-phalangeal joint and 5th metacarpal bone of the toe (A). Image B highlights the fully labelled marker locations and Peak Vertical Force (PVF) exerted (red arrows) once contact is made with the force plates.

Marker visibility proved a common problem when cleaning the gait data, as certain markers were placed in anatomical locations which could easily be obscured by skin, fur or adjacent limbs. Most commonly, axial markers located behind each ear had poor visibility overall, these markers were supposed to provide information about head nods and axial rotation. Furthermore, a single marker was placed at the medial epicondyle of each limb (adjected to the lateral epicondyle) to capture axial rotation of the limb. However, as described by Pascal (2003), in quadrupeds, medially placed markers are often obscured by fur or the contralateral limb. Therefore, minimal data were obtained from these markers in small breeds, overweight animals, or excessively long-haired breeds. Overall, small breed dogs (under 10kgs) and long-haired breeds proved the most challenging when labelling anatomical locations, predominantly due to the markers being very close together, and often overlapping in small breeds. In long-haired breeds, fur was clipped where necessary, however, this was not always feasible in double-coated breeds like Golden retrievers and German Shepherds due to excessive clipping causing skin irritation. Furthermore, some owners did not give written consent to their dogs being clipped, therefore, human hair clips were used where applicable to hold the fur out of the way and allow maximum visibility of the retro-reflective markers. These challenges may limit future analysis of body segment/joint kinematics using the same data. However, analysis of this data was not completed for this Masters project, therefore it would not impact the results presented in this thesis.

4.2.2.1 *Trial Data Analysis*

A total of 15 (out of 25 trotting trials) were fully labelled with the correct anatomical marker locations. This number was deemed feasible due to the time-scale of this Masters project but would also provide adequate data for analysis. No walking trials were labelled during this Masters project, but data is available for future research. Once all 15 trials were labelled, the files were exported from QTM as .tsv files. A total of four .tsv files were exported cumulatively which included spatiotemporal data for each individual marker and data from the three force plates. Peak Vertical Force was used as the kinetic measurement throughout this study as peak values were predominantly used for analysis. Ground Reaction Force (GFR) is used to

measure the entire trace (all values from toe-touch to toe-off) and hence, can be discarded for this analysis.

After data from all forty-four retro-reflective markers were exported via .tsv files for further analysis in MATLAB, the kinematic and kinetic gait events needed to be defined for the same 15 trials containing fully labelled markers. This consisted of manually labelling gait events by determining each foot strike onto and off of each force plate and the laboratory floor. A colour-coding system was used to differentiate each individual limb strike: green (left thoracic limb), blue (right thoracic limb), yellow (left pelvic limb) and red (right pelvic limb). By determining a step onto and off of the force plate/floor for each limb, data could be collected to provide information on peak vertical force, duty factor, stride length and stride width. However, for kinetic analysis of force plate data, all foot strikes on the plate needed to be "clean", meaning that only one foot could be in contact with a single force plate at any one time. This proved especially challenging for smaller breed dogs with smaller stride lengths and for mobility impaired dogs who compensated by shortening their duty factor and stride length (see below). Initially, any trial that had two feet on the force plate at one time was discarded. However, in some cases, this meant that all trials (and consequently the participant) had to be removed from the trial. Therefore, a technique was developed to determine "unclean" strikes from which an accurate PVF (the metric of interest herein) could be obtained. However, in most cases, this technique proved successful in retaining gait data with moderate mobility impairments, and for smaller dogs where shorter stride lengths led to a high frequency of unclean strikes. Following identification of individual gait events, data was exported from QTM at .mat files containing data for Peak Vertical Forces only.

Specific MATLAB scripts were written for the 3D gait data to extract specific spatiotemporal and kinetic gait metrics from the collected data. MATLAB code was written by Dr Karl Bates and required some editing for each individual dog to update information regarding weight, participant ID number and number of total trials before running the script.

2.8.3.1 *Within Participant Analysis*

The second piece of MATLAB code, also written by Dr Karl Bates, allowed within-participant analysis. For this comparison, dogs were divided into separate categories (healthy and mobility impaired) and then placed into subcategories for mobility impaired (combined thoracic and pelvic lameness, solely thoracic lameness and solely hindlimb lameness).

The new MATLAB script had various functions, for example, extracting individual force plate strikes from individual trials to calculate normalised values (by dividing PVF by body mass) and saving this data within individual folders for each limb. Merged files were then created and data was outputted in box plots containing standard divination (SD) (Figure 4.5).

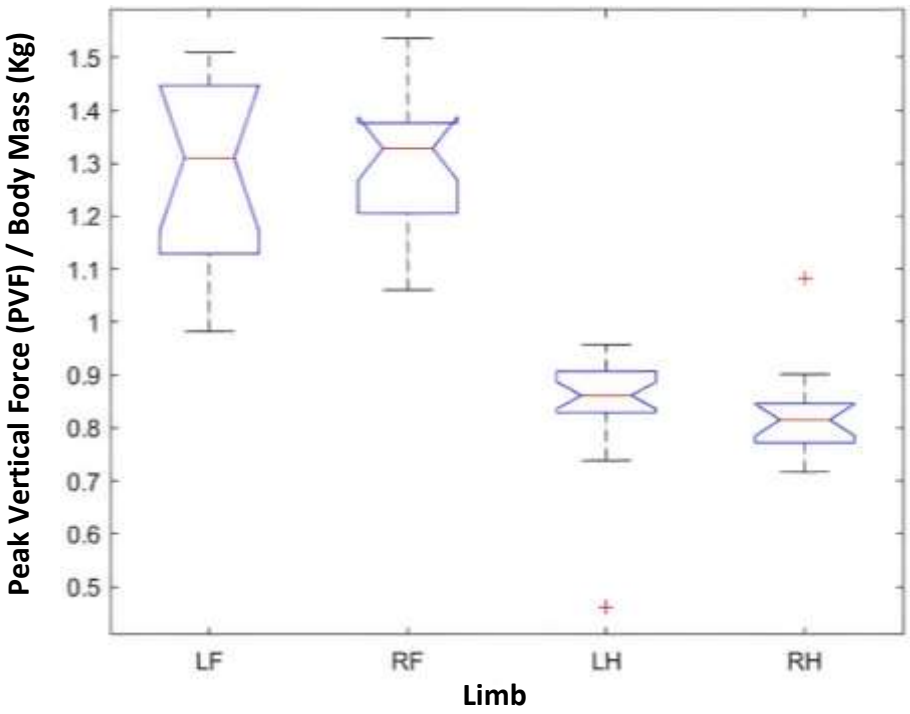

Figure 4. 9. Individual thoracic and pelvic peak vertical force (PVF) normalised by body mass (Kg). Median values are represented by the horizontal red line. Whiskers have end-points that correspond to minimum and maximum values for all feet (left-fore (LF), right-fore (RF), left-hind (LH) and right-hind (RH). Outliers are determined by red crosses.

2.8.3.2 Between Dog Analysis

All between dog group data for 62 dogs (31 mobility impaired and 31 non-mobility impaired) were separated into the relevant mobility folders by MATLAB. Mobility impaired dogs were further subcategorised into thoracic limb mobility impairments, pelvic limb mobility impairments and combined thoracic and pelvic limb mobility impairments.

Statistical comparisons for force plate and spatiotemporal data were undertaken for each dog. Shapiro-Wilk tests were used for every parameter to determine if the data were distributed. The Shapiro-Wilk test (and the number of variables) was used to determine if a Kruskal-Wallis or an Analysis of variance (ANOVA) was the ideal statistical test to run for that data set. Each variable was compared across a limb within each dog, then left and right thoracic and pelvic limb data were combined and compared again to give an overall result for spatiotemporal data and plotted in box plots to enable an 'AllThoracic' and 'AllPelvic' analysis. Another Shapiro-Wilk test is performed to determine if the combined thoracic and pelvic limb data is normally/abnormally distributed to decide if a t-test or Wilcoxon rank-sum test should be performed to determine statistically significant differences between PVF and spatiotemporal parameters between mobility impaired and non-mobility impaired dogs.

SPSS (IBM SPSS Statistics for Windows, Version 27.0) was used to undertake all statistical tests on this dataset. PVF data were non-normally distributed; hence, a Kruskal-Wallis statistical test was used to indicate any statistically significant differences between the PVF in the two categories and subcategories. Significance levels were set to an alpha of $p \leq 0.05$. Non-mobility impaired dogs were coded as '0' in SPSS, and mobility impaired dogs were coded as '1'. Mean, standard error of the mean and standard deviation were also calculated alongside p-values. Using this data, box plots were constructed using Microsoft Excel for non-mobility impaired dogs and mobility impaired dogs (thoracic, pelvic and combined limb impairments), where 'X' represented the median, the whiskers (vertical lines) extended from the ends of the box to show the minimum value and maximum value. Outliers were highlighted by solid blue dots.

ANOVAs were used for spatiotemporal data analysis as data was normally distributed. p-values for cycle time, duty factor, stance time, step width, stride length and swing time were calculated for mobility impaired dogs for comparison against non-mobility impaired dogs. Mean, standard error of the mean and standard deviation were calculated in Microsoft Excel

(Microsoft Corporation, 2019). Mean values for mobility impaired and non-mobility impaired thoracic and pelvic limbs were combined to see if there were statistically significant differences between the means. The thoracic to pelvic limb ratios were also calculated and compared using ANOVA. Again, individual box plots were created to highlight differences between the mobility and non-mobility impaired cohort.

2.8.3.3 Normalising Data

The data for this project was both raw and normalised, depending on the comparisons (i.e. within dog comparison, or between dog comparisons) being made. For the within dog comparison, the spatiotemporal were not normalised since this was not considered necessary to allow the within dog data comparisons. However, the force plate data were normalised for the within dog comparison, this was considered strictly necessary to allow ease when comparing across different dog breeds and body mass, although, this methodology is not considered to affect the overall results.

For this project, all data was normalised due to the large variation in body size and breed and to prevent data from being skewed. Normalised data was obtained by the following calculation: speed was normalised into a Froude number (Froude = speed x speed) / (gravity x leg length). Froude numbers as considered dimensionless, and thus, have no units. Spatial parameters (step width and stride length) were normalised by leg length, this is consistent with the literature on Froude numbers (Alexander, 1976) where relative stride length is stride length divided by hip height (hip height = limb length), again these units are dimensionless. Temporal gait variables were normalised by the square of the ratio of each subject limb length to gravitational acceleration, the normalisation metric = sqrt (BH/g) and the normalised temporal parameter = raw parameter/normalisation metric, this calculation followed Hof, 1996) and is equivalent to the Froude number for temporal parameters, therefore, these results are dimensionless values and have no units.

4.3 Results

4.3.1 *Within-Participant Kinetic (Force Plate) Data*

4.3.1.1 *Non-Mobility impaired dogs*

Raw, normalised data for the 31 non-mobility impaired dogs showed minimal changes between left thoracic (LT) and right thoracic (RT) peak-vertical force (PVF) (expressed as Newtons per kilograms (N/Kg)): LT = 1.25N/Kg (0.14) ± 0.77 and RT = 1.21N/Kg (0.13) ± 0.67. Left pelvic (LP) and right pelvic (RP) PVF values also showed minimal deviation from one another in the dataset: LP = 0.86N/Kg (0.09) ± 0.54 and RP = 0.87N/Kg (0.1) ± 0.63. None of the 31 non-mobility impaired dogs had statistically significance differences between the PVF LT versus PVF RT, or PVF LP versus PVF RP ($p=<0.05$; Figure 4.6).

A Kruskal-Wallis or ANOVA test was undertaken following the Shapiro-Wilk test on all non-mobility impaired dogs. The above stasticial tests allow evaluation of the statistical significance between individual limbs. The p-values (<0.05) for peak vertical force (PVF) of non-mobility impaired dogs can be found in corresponding comparisons are shown in Table 4.1.

Participant ID Number	Left Thoracic versus Right Thoracic Limb	Left Thoracic versus Left Pelvic Limb	Right Thoracic versus Right Pelvic Limb	Left Pelvic versus Right Pelvic Limb	Chosen Statistical Test
3	0.441	0.0002	0.006	0.722	Kruskal-Wallis
6	0.861	<0.0001	0.0001	0.997	Kruskal-Wallis
10	0.689	0.0009	<0.0001	0.365	Kruskal-Wallis
11	0.043	<0.0001	0.097	0.998	Kruskal-Wallis
13	0.995	<0.0001	<0.0001	0.699	Kruskal-Wallis
14	0.996	<0.0001	<0.0001	0.661	Kruskal-Wallis
15	0.080	<0.0001	<0.0001	0.606	Kruskal-Wallis
19	<0.0001	<0.0001	0.0004	0.973	ANOVA
22	0.996	<0.0001	<0.0001	0.389	ANOVA
23	0.561	<0.0001	<0.0001	0.407	ANOVA
26	0.173	<0.0001	0.0001	0.953	Kruskal-Wallis
30	0.998	<0.0001	0.0002	0.327	Kruskal-Wallis
33	0.719	0.0002	<0.0001	0.978	Kruskal-Wallis
35	0.012	<0.0001	<0.0001	0.998	ANOVA
38	0.999	<0.0001	<0.0001	0.658	ANOVA
40	0.999	<0.0001	0.0001	0.999	Kruskal-Wallis
41	0.444	0.289	0.422	0.439	ANOVA
45	0.384	<0.0001	<0.0001	0.356	ANOVA
48	0.862	0.0004	0.0001	0.531	Kruskal-Wallis
52	0.831	<0.0001	<0.0001	0.859	ANOVA
56	0.998	<0.0001	<0.0001	0.999	Kruskal-Wallis
58	0.887	<0.0001	0.016	0.547	Kruskal-Wallis
59	0.173	0.276	0.648	0.761	ANOVA
60	0.741	<0.0001	<0.0001	0.860	Kruskal-Wallis
61	0.457	<0.0001	<0.0001	0.978	Kruskal-Wallis
62	0.985	<0.0001	<0.0001	0.366	ANOVA
65	0.091	0.126	<0.0001	0.273	Kruskal-Wallis
66	0.990	<0.0001	<0.0001	0.915	ANOVA
67	0.230	<0.0001	0.001	0.813	Kruskal-Wallis
68	0.983	<0.0001	<0.0001	0.713	Kruskal-Wallis

Table 4. 11. This table shows the p-values for the peak vertical force (PVF) for individual limbs for all dogs with no detected mobility impairments. The significance level was set to p=<0.05) in non-mobility impaired dogs. For normally distributed data, and p-values >0.05, Analysis of variance (ANOVA) was used.

Statistical significance between left thoracic versus left pelvic limb were recovered in twenty-nine out of 31 non-mobility dogs (all of which had higher thoracic PVF values compared to pelvic PVF values).

4.3.1.1.1 *Individual Dog Differences*

Two remaining dogs (PID 41 and 59) yielded a non-statistically significant difference, $p>0.05$ ($p= 0.29$ and 0.28, respectively). Both of these dogs had very little difference in mean left thoracic and pelvic PVF (PID 41 = 0.69 and 0.64 respectively, and PID 59 = 0.73 and 0.67, respectively for LT and LP PVF). These same two dogs, PID 41 and 59, also had no statistical significance between the right thoracic versus right pelvic limbs ($p= 0.42$ and 0.64), (RT = 0.69 versus RP = 0.66) as showed in Table 4.1 and Figure 4.6. PID 11 had PVF values $p<0.05$ for all limbs, except when comparing right thoracic and right pelvic limbs, where $p = 0.1$ (RT = 0.85 versus RP = 0.79).

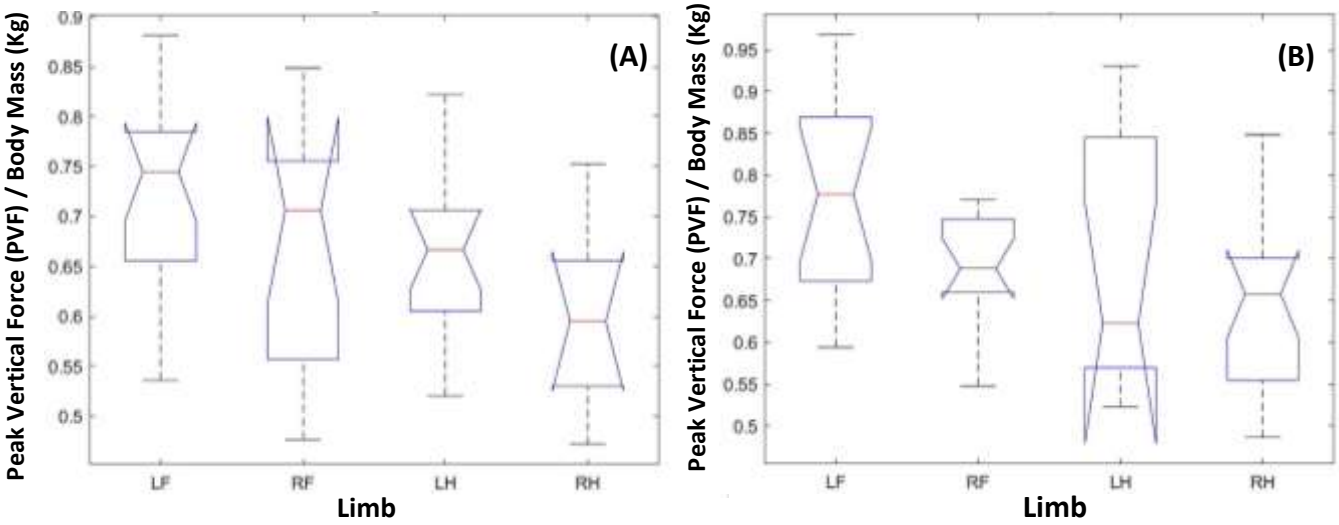

Figure 4. 6. Individual thoracic and pelvic peak vertical force (PVF) normalised by body mass (Kg) for Participants 41 (A) and 59 (B). Median values are represented by the horizontal red line. Whiskers have end-points that correspond to minimum and maximum values for all feet (left-fore (LF), right-fore (RF), left-hind (LH) and right-hind (RH).

4.3.1.1.2 *Peak Vertical Force*

All dogs had higher PVF for thoracic limbs compared to PVF for pelvic limbs (p=<0.05). Thoracic = 2.46N/Kg (0.26) ± 1.44 and pelvic = 1.74N/Kg (0.21) ± 1.17. However, there was a large magnitude across the non-mobility impaired cohort: range for PVF LT = 4.38N/Kg, PVF LP = 3.12N/Kg, PVF RT = 3.63N/Kg, PVF RP = 3.74N/Kg. Z values are used due to the large sample size to compute the approximate *p*-value of the test. All Z values in this data set are above 1.96, and are, therefore, statistically significant (Sim and Wright, 2005).

Participant ID Number	Ranksum Value	Z value	p-value	Chosen Statistical Test
3	728	5.0293	<0.0001	Wilcoxon rank-sum
6	1865	6.1292	<0.0001	Wilcoxon rank-sum
10	2109	7.0418	<0.0001	Wilcoxon rank-sum
11	1336	5.0542	<0.0001	Wilcoxon rank-sum
13	1424.5	6.6794	<0.0001	Wilcoxon rank-sum
14	1820	7.1354	<0.0001	Wilcoxon rank-sum
15	2367	7.1832	<0.0001	Wilcoxon rank-sum
19	706	4.941	<0.0001	Wilcoxon rank-sum
22	1749	7.1294	<0.0001	Wilcoxon rank-sum
23	1617	6.9216	<0.0001	Wilcoxon rank-sum
26	1582	6.8954	<0.0001	Wilcoxon rank-sum
30	1488	6.8122	<0.0001	Wilcoxon rank-sum
33	1272.5	6.4249	<0.0001	Wilcoxon rank-sum
35	1349.5	6.4165	<0.0001	Wilcoxon rank-sum
38	1365	6.6456	<0.0001	Wilcoxon rank-sum
40	1245	6.5121	<0.0001	Wilcoxon rank-sum
41	844	2.1864	0.028	Wilcoxon rank-sum
45	1079	6.2929	<0.0001	Wilcoxon rank-sum
48	805	5.7999	<0.0001	Wilcoxon rank-sum
52	852	5.863	<0.0001	Wilcoxon rank-sum
56	1274	6.5004	<0.0001	Wilcoxon rank-sum
58	1160	6.2798	<0.0001	Wilcoxon rank-sum
59	1038	2.373	0.017	Wilcoxon rank-sum
60	2631	7.5141	<0.0001	Wilcoxon rank-sum
61	1820	7.1354	<0.0001	Wilcoxon rank-sum
62	1189	6.0098	<0.0001	Wilcoxon rank-sum
65	1174	5.6899	<0.0001	Wilcoxon rank-sum
66	759	5.6628	<0.0001	Wilcoxon rank-sum
67	2756	7.5943	<0.0001	Wilcoxon rank-sum
68	2420	7.6932	<0.0001	Wilcoxon rank-sum

Table 4. 12. A table to show the Wilcoxon rank-sum values for all non-mobility impaired dogs. Wilcoxon rank-sum tests were used on all non-mobility impaired dogs to compare thoracic and pelvic limb peak vertical force (PVF) values. A Wilcoxon rank-sum test was used if data was considered to be non-normally distributed. For normally distributed data (and p-values >0.05), a t-test was used.

Following analysis of individual limbs, left and right values were combined to allow comparison of overall thoracic versus pelvic limb PVFs. The results of Wilcoxon rank-sum tests applied to this data are presented in Table 4.2 below.

4.3.1.2 *Mobility impaired Dogs*

Raw data for the 31 mobility impaired dogs showed minimal changes between left thoracic (LT) and right thoracic (RT) peak-vertical force (PVF): LT = 1.24N/Kg (0.11) ± 0.43 and RT = 1.23N/Kg (0.11) ± 0.42. Left pelvic (LP) and right pelvic (RP) PVF values also showed minimal deviation from each other: LP = 0.85N/Kg (0.07) ± 0.28 and RP = 0.84N/Kg (0.06) ± 0.25.

A Kruskal-Wallis or ANOVA test was also undertaken, following the Shapiro-Wilk test for the mobility impaired group, to test for statistically significant differences in PVFs between individual limbs. The mobility impaired group was further sub-divided into 'combined' (where both thoracic and pelvic limbs were affected) 'thoracic limb' (where only thoracic limbs were affected) and pelvic limb (where only pelvic limbs were affected). Twenty-seven dogs showed no statistical difference between LT versus RT limbs, and LP versus RP limbs ($p=<0.05$; Table 4.3). Twenty-four dogs showed statistically significant differences between LT versus LP limb, and RT versus RP limb (all of which had higher thoracic PVF values compared to pelvic PVF values) ($p=<0.05$).

	Participant ID Number	Left Thoracic versus Right Thoracic Limb	Left Thoracic versus Left Pelvic Limb	Right Thoracic versus Right Pelvic Limb	Left Pelvic versus Right Pelvic Limb
Combined Mobility Impairments	1	0.266	<0.0001	0.001	0.397
	8	0.270	<0.0001	<0.0001	0.195
	9	0.991	0.0001	<0.0001	0.952
	31	0.999	<0.0001	<0.0001	0.499
	34	0.419	<0.0001	<0.0001	0.685
	50	0.156	0.954	0.014	0.547
	54	0.988	<0.0001	0.0001	0.947
	7	0.030	0.025	0.0008	0.0004
Thoracic Limb Mobility Impairments	32	0.071	<0.0001	0.0005	0.698
	36	0.205	0.370	0.0005	0.964
	37	0.785	<0.0001	<0.0001	0.961
	46	0.353	<0.0001	0.015	0.558
	51	0.470	<0.0001	0.007	0.979
	55	0.998	<0.0001	<0.0001	0.516
	57	0.026	<0.0001	<0.0001	0.939
Pelvic Limb Mobility Impairments	5	0.370	0.0003	<0.0001	0.636
	12	0.281	<0.0001	0.003	0.586
	24	0.995	<0.0001	<0.0001	0.146
	27	0.956	<0.0001	<0.0001	0.995
	28	0.854	<0.0001	0.0005	0.884
	29	0.993	<0.0001	<0.0001	0.577
	39	0.362	<0.0001	<0.0001	0.927
	42	0.719	0.0003	0.0004	0.461
	43	0.973	<0.0001	0.001	0.997
	47	0.888	<0.0001	<0.0001	0.460
	49	0.641	0.0004	0.003	0.912
	53	0.999	<0.0001	<0.0001	0.146
	63	0.465	0.194	<0.0001	0.188
	64	0.392	0.083	<0.0001	0.661

Table 4. 13. This table shows the p-values for the peak vertical force (PVF) for individual limbs for all dogs with no detected mobility impairments. Individual thoracic and pelvic limbs in mobility impaired dogs were compared against one another to determine statistical significance using an Analysis of variance (ANOVA). The mobility impaired cohort were further divided into thoracic, pelvic and combined mobility impairments to highlight any differences between groups.

4.3.1.2.1 *Individual Dog Differences*

PID 50 and PID 7 both had combined mobility problems and had no significance when LT and LP limbs, and RT and RP limbs were compared (as shown in Table 4.3 and Figure 4.7). Similarly, both of these dogs had little differences in mean LT and LP PVF (PID 50 = 0.68 and 0.62, and PID 7 = 0.82 and 0.72, respectively) and RT and RP PVF (PID 7 = 0.85 and 0.78, and PID 7 = 0.83 and 0.78, respectively). Additionally, PID 7 was the only dog that showed statistically significant differences between LT versus RT limbs, and LP and RP limbs. PID 36 had a unilateral left thoracic limb mobility impairment identified on veterinary clinical examination and was the only dog that showed no significant difference between LT versus LP limb (p=0.37). Consequently, there were minimal differences between LT and LP mean PVF (0.96 and 0.91, respectively). PID 57 also had a unilateral thoracic limb impairment and was the only dog who had p<0.05 for LT versus RT PVF. Mean PVF values for LT and RT limbs were 0.95 and 0.72, respectively).

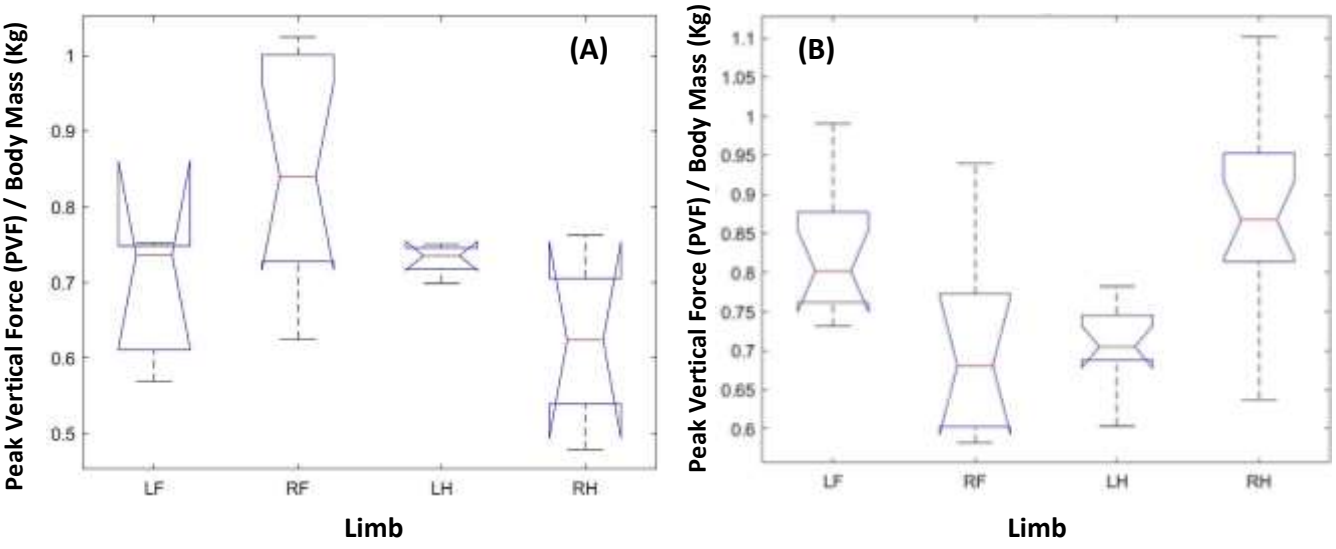

Figure 4. 7. Individual thoracic and pelvic peak vertical force (PVF) normalised by body mass (Kg) for Participants 7 (A) and 50 (B). Median values are represented by the horizontal red line. Whiskers have end-points that correspond to minimum and maximum values for all feet (left-fore (LF), right-fore (RF), left-hind (LH) and right-hind (RH).

PID 63 and PID 64 both had pelvic limb lameness identified on veterinary clinical examination, with PID 64 having a bilateral pelvic limb mobility impairment. Both dogs were the only participants to have no statistically significant differences between LT and LP PVF (Figure 4.8 and Table 4.3). PID 63 had higher mean thoracic PVF values compared to mean pelvic PVF, however, the mean variability was minimal. PID 63 had very low variability between thoracic and pelvic PVF for left and right limbs compared to overall values. LT and LP PVF values were 0.60 and 0.49, respectively.

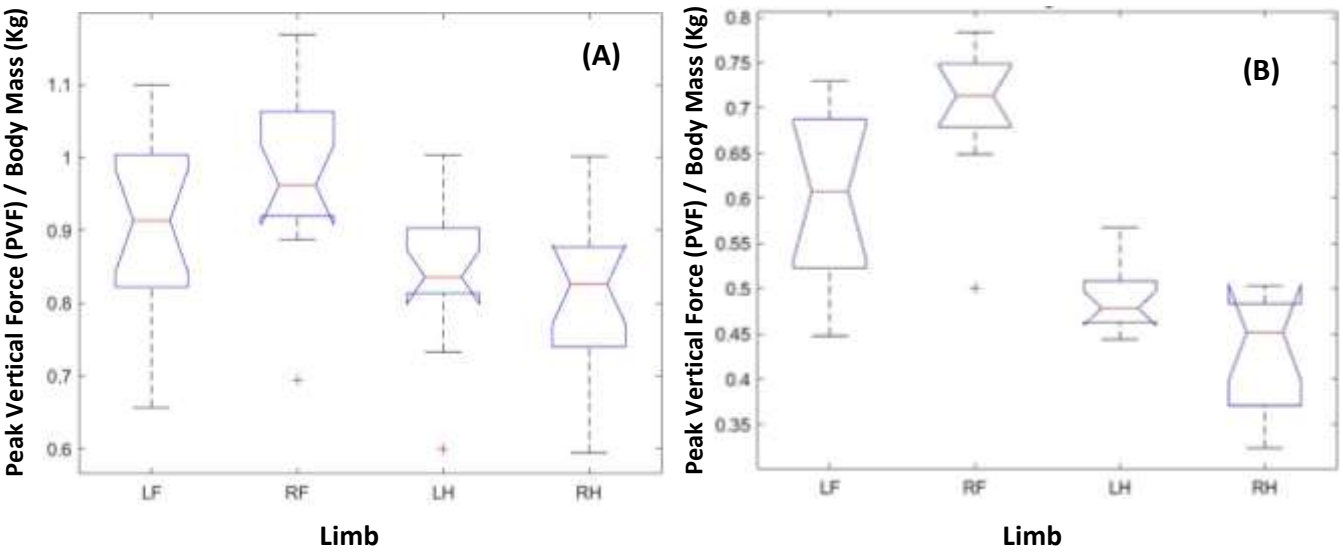

Figure 4. 8. Individual thoracic and pelvic peak vertical force (PVF) normalised by body mass (Kg) for Participants 63 (A) and 64 (B). Median values are represented by the horizontal red line. Whiskers have end-points that correspond to minimum and maximum values for all feet (left-fore (LF), right-fore (RF), left-hind (LH) and right-hind (RH). Outliers are determined by red crosses.

Similar to non-mobility impaired dogs, all PVF values for individual limbs were combined together to give an overall thoracic and pelvic limb PVF values. Wilcoxon rank-sum tests were used to determine the significance among the three sub-groups within the mobility impaired cohort. Data are presented in Table 4.4 below.

	Participant ID Number	Ranksum Value	Z value	p-value
Combined Mobility Impairments	1	2220	7.428	<0.0001
	8	1751	7.083	<0.0001
	9	1488	6.754	<0.0001
	31	1488	6.812	<0.0001
	34	943	5.839	<0.0001
	50	223	2.087	0.036
	54	1080	6.236	<0.0001
Thoracic Limb Mobility Impairments	7	504	0.794	0.427
	32	1305	6.527	<0.0001
	36	1338.5	3.880	<0.0001
	37	1820	7.135	<0.0001
	46	1311	6.224	<0.0001
	51	781	5.710	<0.0001
	55	759	5.662	<0.0001
	57	1386	6.469	<0.0001
Pelvic Limb Mobility Impairments	5	2447.5	7.570	<0.0001
	12	1855	7.188	<0.0001
	24	2757	7.652	<0.0001
	27	1550	6.841	<0.0001
	28	1395	6.701	<0.0001
	29	1474	6.520	<0.0001
	39	1209	6.329	<0.0001
	42	756	5.777	<0.0001
	43	1103	6.173	<0.0001
	47	900	5.998	<0.0001
	49	875	5.097	<0.0001
	53	1071	6.076	<0.0001
	63	1162	6.355	<0.0001
	64	806	4.994	<0.0001

Table 4. 14. A table to show the Wilcoxon rank-sum values for all non-mobility impaired dogs. Both thoracic and pelvic limbs were compared against each another to determine statistical significance ($p \leq 0.05$) in the mobility impaired category. Values were further divided into thoracic, pelvic and combined mobility impairments.

Thirty dogs showed statistical significance between the peak-vertical force (PVF) between thoracic versus pelvic limbs (p=<0.05). Subsequently, all these thirty dogs had Z values above 1.96, and are therefore, considered statistically significant (Sim and Wright, 2005). PID 7 was the only dog with p=>0.05, and thus, not significant. Consequently, PID 7 was also the only participant with a Z value <1.96.

In all cases (regardless of mobility impairment), all dogs had higher a PVF on thoracic limbs compared to pelvic limbs: thoracic = 2.27N/Kg (0.22) ± 0.85 and pelvic = 1.47N/Kg (0.15) ± 0.53. However, there was a large magnitude across the mobility impaired cohort: range for PVF LT = 4.26N/Kg, PVF LP = 3.22N/Kg, PVF RT = 4.43N/Kg, PVF RP = 3.65N/Kg.

4.3.2 *Within-Participant Kinematic (Spatiotemporal) Data*

4.3.2.1 *Non-Mobility impaired Dogs*

A Kruskal-Wallis or ANOVA test (depending on normality of data distribution) was undertaken following the Shapiro-Wilk test, allowing evaluation of the statistical significance between spatiotemporal kinematic variables (cycle time, duty factor, stance time, step width, stride length and swing time) for individual limbs of all 31 dogs with known mobility impairments. The p-values ($p=<0.05$) of these tests for corresponding comparisons are shown in Table 4.5, where LT/RT means left/right thoracic limb and LP/RP signifies left/right pelvic limb. The participant identification (PID) number is shown on the left column to maintain dog anonymity. The definition for each of the spatiotemporal parameters can be found in the 'List of Abbreviations' on pages 11-13.

Thirty dogs had no statistical significance for normalised **cycle time** (this data is non-normalised). Mean cycle times for LT and RT were 0.38s (0.02) ± 0.16, and 0.37s (0.03) ± 0.16. These values did not change for LP and RP limbs. PID 58 was the only dog that yielded a $p<0.05$ for LT versus LP, and RT versus RP. Cycle time values for this dog were 0.51s and 0.47s, respectively. There were no significant differences between LT versus RT, and LP versus RP cycle time. All thirty-one dogs had a higher **duty factor** for thoracic limbs compared to pelvic limbs. The majority of dogs had $p=<0.05$ for LT versus LP, and RT versus RP limbs for duty factor. Average normalised values for LT and RT duty factor was 0.39s (0.02) ± 0.14, and 0.41s (0.01) ± 0.17, respectively. Similarly, mean values for LP and RP limbs were 0.35s (0.2) ± 0.14 and 0.36s (0.15) ± 0.01. PID 14, 60 and 65 were the only participants to retrieve significant values for duty factor when LT was compared against RT. PID 10, 35, 38 and 60 all yielded $p<0.05$ for LP versus RP values.

Stance times were generally higher for thoracic limbs, compared to pelvic limbs (0.19s and 0.18s, compared to 0.16s and 0.15s, respectively). There were minimal variations between stance time, and most participants had no statistically significant differences between LT and LP, and RT and RP values. All thirty dogs had no statistically significant differences between LT and RT, and LP and RP stance times. Only PID 56 had statistically significant values between thoracic and pelvic step widths. There was minimal variability in non-normalised **stride length** values; LT, RT, LP and RP all have values of ~0.74m (0.05) ± 0.31. However, PID 58 had p= 0.03 and stride length values >1 for both thoracic and pelvic limbs. **Swing time** was generally higher in pelvic limbs (LP = 0.22s (0.01) ± 0.09, RP = 0.21s (0.01) ± 0.09) compared to thoracic limbs (LT = 0.20s (0.01) ± 0.08, RT = 0.19s (0.01) ± 0.08). Two dogs (PID 14 and PID 65) had significant differences for LT versus RT limbs. Similarly, PID 35 and 58 had $p<0.05$ for LP versus RP limbs.

Following analysis of individual limbs, thoracic and pelvic spatiotemporal values were compared against each other to see if there were statistical differences between these variables ($p<0.05$). t-tests and Wilcoxon rank-sum tests were used to determine the statistical significance levels among healthy individuals. Data is presented in Figure 4.6.

	Cycle Time (p-values)				Duty Factor (p-values)				Stance Time (p-values)				Step Width (p-values)		Stride Length (p-values)				Swing Time (p-values)			
	LT vs RT	LT vs LP	RT vs RP	LP vs RP	LT vs RT	LT vs LP	RT vs RP	LP vs RP	LT vs RT	LT vs LP	RT vs RP	LP vs RP	Thoracic Limbs	Pelvic Limbs	LT vs RT	LT vs LP	RT vs RP	LP vs RP	LT vs RT	LT vs LP	RT vs RP	LP vs RP
PID																						
3	0.903	0.984	0.989	0.999	1.000	***<0.0001***	***<0.0001***	0.399	1.000	***0.001***	***<0.0001***	0.851	0.121	0.563	0.900	0.556	0.997	0.849	0.958	***<0.0001***	***<0.0001***	0.268
6	0.854	1.000	0.943	0.540	0.417	0.294	***0.003***	0.998	0.586	0.846	0.098	0.997	0.250	0.424	0.767	0.988	0.999	0.859	0.996	0.134	0.004	0.505
10	0.975	0.997	0.999	0.959	0.986	0.063	0.946	***0.005***	0.979	0.311	0.974	0.054	0.932	***<0.0001***	1.000	0.992	0.979	0.908	1.000	0.253	0.994	0.437
11	0.979	1.000	0.814	0.983	0.493	***<0.0001***	***<0.0001***	1.000	0.135	***0.002***	***<0.0001***	0.983	0.376	0.058	0.999	0.902	0.977	0.969	0.124	***<0.0001***	***<0.0001***	0.960
13	0.990	0.692	0.399	0.858	0.887	***<0.0001***	***<0.0001***	0.865	0.740	***<0.0001***	***<0.0001***	0.850	0.936	0.096	0.996	0.580	0.767	0.952	0.955	***<0.0001***	***<0.0001***	0.996
14	0.945	0.880	0.913	0.206	***0.049***	***<0.0001***	***<0.0001***	0.842	0.383	***<0.0001***	***<0.0001***	0.891	0.070	***0.046***	0.952	0.806	0.999	0.409	***0.075***	***<0.0001***	***<0.0001***	0.103
15	0.995	0.819	0.977	0.890	0.330	***<0.0001***	***<0.0001***	0.865	0.966	***<0.0001***	***<0.0001***	0.872	0.325	0.797	0.519	0.951	0.302	0.802	0.805	***<0.0001***	***<0.0001***	0.789
19	0.767	0.946	0.954	1.000	0.649	***0.009***	***<0.0001***	0.673	0.404	***0.012***	***<0.0001***	0.527	0.783	0.264	0.859	1.000	1.000	0.816	0.347	0.070	***<0.0001***	0.550
22	0.932	0.999	0.785	0.997	0.936	***<0.0001***	***<0.0001***	0.583	0.897	***<0.0001***	***<0.0001***	0.786	0.223	0.853	0.877	0.746	0.925	0.817	0.871	***<0.0001***	***<0.0001***	0.950
23	0.993	1.000	0.978	0.998	0.994	***<0.0001***	***<0.0001***	0.355	0.987	***<0.0001***	***<0.0001***	0.583	0.436	0.484	0.963	0.920	0.998	0.989	0.992	***<0.0001***	***<0.0001***	0.322
26	1.000	0.957	0.996	0.981	0.304	***<0.0001***	***<0.0001***	0.963	0.098	***<0.0001***	***<0.0001***	0.954	***0.026***	0.155	1.000	0.917	0.945	1.000	0.037	***<0.0001***	***<0.0001***	0.832
30	0.975	0.898	0.998	1.000	0.808	***0.006***	***<0.0001***	0.999	0.926	***0.001***	***<0.0001***	0.985	***0.032***	0.431	1.000	0.852	0.998	0.737	0.925	***0.001***	***<0.0001***	0.978
33	0.972	0.940	0.878	0.816	0.696	0.143	***<0.0001***	0.478	0.867	***0.016***	***<0.0001***	0.857	***<0.0001***	0.443	0.805	1.000	0.986	0.951	0.615	0.755	***0.005***	0.672
35	1.000	0.803	0.494	0.977	0.193	***<0.0001***	***<0.0001***	***<0.0001***	0.532	***<0.0001***	***<0.0001***	0.136	***0.009***	0.091	0.992	0.256	0.164	1.000	0.330	***<0.0001***	***<0.0001***	***0.014***
38	0.974	0.988	0.444	0.872	0.910	0.583	***<0.0001***	***0.015***	0.994	0.409	***0.017***	0.644	0.723	0.910	0.608	0.970	0.664	0.954	0.839	0.511	***0.001***	0.335
40	0.630	0.276	0.917	1.000	1.000	***<0.0001***	***<0.0001***	0.875	0.840	***<0.0001***	***<0.0001***	0.325	0.457	***0.047***	0.976	0.993	0.826	0.998	0.940	***<0.0001***	***<0.0001***	0.989
41	1.000	0.884	0.971	0.996	0.887	0.824	0.868	0.803	0.998	0.997	1.000	0.966	0.545	0.108	0.936	0.938	0.988	0.990	0.628	0.991	0.500	0.960
45	1.000	1.000	0.999	0.999	0.939	0.355	***0.005***	0.657	0.959	0.431	***0.098***	0.991	0.561	0.073	0.693	0.954	0.989	0.552	0.986	0.717	0.067	0.717
48	0.862	0.999	0.996	0.981	0.771	***<0.0001***	***<0.0001***	0.994	0.815	***0.002***	***<0.0001***	0.956	0.362	***<0.0001***	1.000	0.625	0.414	0.979	0.999	***0.003***	***0.001***	1.000
52	0.407	***0.0001***	***0.0001***	0.770	0.407	***<0.0001***	***<0.0001***	0.770	0.744	***<0.0001***	***<0.0001***	0.978	0.844	0.465	0.963	0.898	0.865	0.979	0.839	***<0.0001***	0.071	0.847
56	0.999	0.745	0.494	0.995	0.992	***<0.0001***	***0.001***	0.842	0.963	***0.001***	0.182	0.115	***0.001***	***<0.0001***	0.988	0.996	0.967	0.985	0.993	***<0.0001***	***<0.0001***	0.626
58	0.999	0.070	0.790	0.333	0.700	***0.001***	***<0.0001***	0.234	0.984	***<0.0001***	***<0.0001***	0.119	0.684	0.188	0.760	***0.035***	0.313	0.212	0.867	0.052	***<0.0001***	***0.029***
59	0.998	0.875	0.988	0.996	0.965	0.065	0.948	0.511	0.233	***0.009***	0.871	0.167	***<0.0001***	0.745	0.997	0.546	0.677	0.986	0.977	0.010	0.957	0.141
60	0.957	0.404	0.990	0.531	***0.036***	***<0.0001***	***<0.0001***	***0.007***	0.319	***<0.0001***	***<0.0001***	0.431	0.054	***0.017***	0.876	0.685	0.557	0.773	0.478	***<0.0001***	***<0.0001***	0.221
61	0.999	0.910	0.828	0.989	0.284	***<0.0001***	***<0.0001***	0.178	0.188	***<0.0001***	***<0.0001***	0.181	0.191	0.849	0.997	0.999	0.998	0.959	0.211	***<0.0001***	***<0.0001***	0.487
62	0.982	0.351	0.990	0.765	0.089	0.935	0.026	1.000	0.113	0.995	0.117	0.987	***0.031***	0.777	0.989	0.988	0.904	0.554	0.160	0.397	***0.015***	0.916
65	0.990	0.046	***0.033***	0.983	***0.047***	***<0.0001***	***<0.0001***	0.016	0.167	***<0.0001***	***<0.0001***	0.007	0.599	0.613	0.991	0.550	0.462	0.974	***0.015***	***0.041***	***0.008***	0.054
66	0.636	0.757	0.518	0.646	1.000	***<0.0001***	***<0.0001***	0.562	1.000	0.109	***0.002***	0.570	0.223	0.550	0.658	0.948	0.982	0.996	0.786	***0.001***	0.093	0.961
67	0.992	0.984	0.906	0.876	0.259	***<0.0001***	***<0.0001***	0.401	0.123	***0.001***	***<0.0001***	0.905	0.451	0.261	0.999	1.000	0.971	0.993	0.199	***<0.0001***	***<0.0001***	0.393
68	0.981	0.743	0.977	0.727	0.876	***0.008***	0.445	0.801	0.967	***0.010***	0.675	0.406	0.477	0.717	0.539	0.183	1.000	0.945	0.902	0.022	0.176	1.000

Table 4. 15. A table to show the p-values for each spatiotemporal parameter for non-mobility impaired dogs. Significance level was set of $p<0.05$. p-values were obtained by either Analysis of variance (ANOVA) or Kruskal-Wallis statistical test. Statically significant values for non-mobility impaired dogs are highlighted in **bold** and *italics*.

	Cycle Time (p-values)	Duty Factor (p-values)	Stance Time (p-values)	Step Width (p-values)	Stride Length (p-values)	Swing Time (p-values)
	Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs
PID						
3	0.962	***<0.0001***	***<0.0001***	0.229	0.430	***<0.0001***
6	0.668	***<0.0001***	***0.031***	0.541	0.963	***<0.0001***
10	0.818	0.171	0.344	***0.024***	0.607	0.134
11	0.478	***<0.0001***	***<0.0001***	***0.005***	0.108	***<0.0001***
13	0.063	***<0.0001***	***<0.0001***	0.345	0.571	***<0.0001***
14	0.936	***<0.0001***	***<0.0001***	0.232	0.387	***<0.0001***
15	0.362	***<0.0001***	***<0.0001***	***0.001***	0.971	***<0.0001***
19	1	***<0.0001***	***<0.0001***	***0.016***	***0.010***	***<0.0001***
22	0.451	***<0.0001***	***<0.0001***	***<0.0001***	0.738	***<0.0001***
23	0.803	***<0.0001***	***<0.0001***	***0.016***	0.397	***<0.0001***
26	0.617	***<0.0001***	***<0.0001***	0.860	0.616	***<0.0001***
30	0.541	***<0.0001***	***<0.0001***	0.113	0.824	***<0.0001***
33	0.900	***<0.0001***	***<0.0001***	***0.016***	***0.006***	***0.002***
35	0.101	***<0.0001***	***<0.0001***	0.218	***0.300***	***<0.0001***
38	0.204	***<0.0001***	***0.002***	0.583	0.420	***0.001***
40	0.192	***<0.0001***	***<0.0001***	0.227	0.519	***<0.0001***
41	0.410	0.958	***0.948***	0.262	0.524	0.212
45	0.894	***0.002***	***0.009***	0.163	0.054	***0.013***
48	0.982	***<0.0001***	***<0.0001***	***0.007***	0.277	***<0.0001***
52	0.413	***<0.0001***	***<0.0001***	***0.030***	0.873	***<0.0001***
56	0.089	***<0.0001***	***<0.0001***	0.850	***0.002***	***<0.0001***
58	***0.020***	***<0.0001***	***<0.0001***	0.959	0.095	***<0.0001***
59	0.439	***0.031***	***<0.0001***	0.325	0.092	***0.009***
60	0.371	***<0.0001***	0.095	***0.009***	0.966	***<0.0001***
61	0.285	***<0.0001***	***<0.0001***	0.816	0.800	***<0.0001***
62	0.166	***0.018***	***<0.0001***	***0.001***	0.053	***0.001***
65	***<0.0001***	***<0.0001***	0.105	0.395	***<0.0001***	***<0.0001***
66	0.782	***<0.0001***	***<0.0001***	0.908	0.732	***<0.0001***
67	0.777	***<0.0001***	***<0.0001***	0.065	0.133	***<0.0001***
68	0.996	***0.001***	***0.003***	***<0.0001***	0.145	***0.001***

Table 4. 16. A table to show the p-values for each spatiotemporal parameter for n=31 non-mobility impaired dogs when individual thoracic and pelvic values were combined. Significance level was set of p<0.05. p-values were obtained by either T-test or Wilcoxon Rank-Sum statistical test. Statistically significant values for non-mobility impaired dogs are highlighted in **bold** and *italics*.

4.3.2.1.1 *Cycle Time, Duty Factor and Stance Time*

The majority of participants showed no statistical difference between non-normalised thoracic and pelvic **cycle times**. There was relatively little variation within thoracic and pelvic cycle times overall (0.38s (0.01) ± 0.03, and 0.38s (0.01) ± 0.03, respectively). PID 65 was the only dog that had a p-value >0.05 and had higher thoracic cycle time values compared to pelvic values. Most participants had significant differences between non-normalised thoracic and pelvic **duty factors**; thoracic values were generally higher than pelvic values (0.40s (0.007) ± 0.02, compared to 0.35s (0.01) ± 0.02). Two dogs (PID 10 and PID 41) yielded p-values >0.05 and therefore, did not exhibit statistically different duty factors between thoracic and pelvic limbs (Figure 4.9 and Table 4.6). Twenty-nine dogs had statistically significant differences between non-normalised thoracic and pelvic **stance times**, where thoracic limb values were higher than pelvic limb values (0.19s (0.006) ± 0.02) and 0.17s (0.006) ± 0.02).

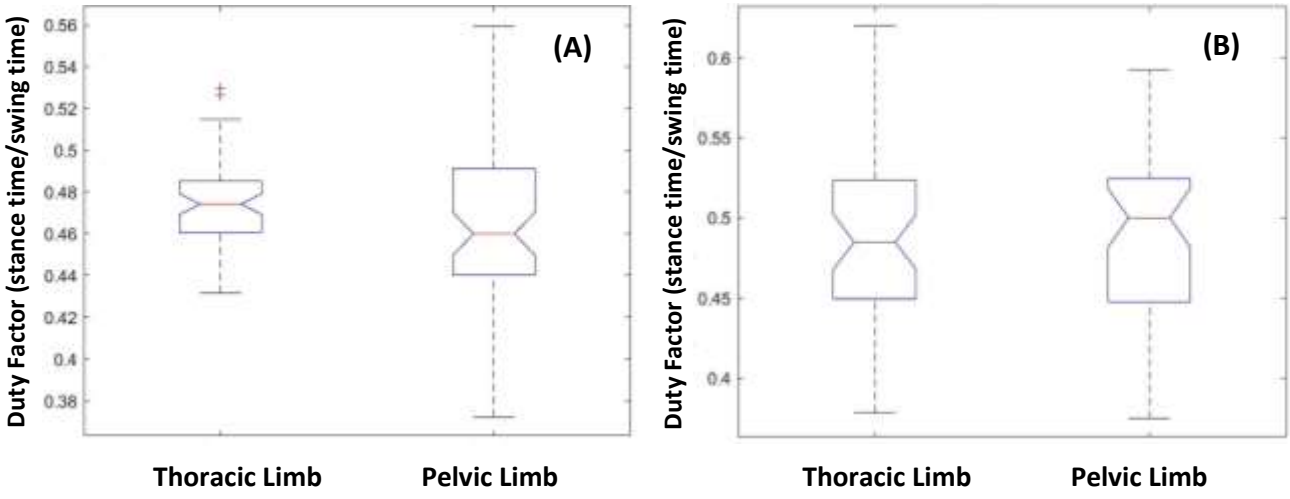

Figure 4. 9. Non-normalised duty factor (stance time/swing time) values for thoracic and pelvic limbs for Participants 10 (A) and 41 (B). Median values are represented by the horizontal red line. Whiskers have end-points that correspond to minimum and maximum values thoracic and pelvic values. Outliers are determined by red crosses.

4.3.2.1.2 Step Width, Stride Length and Swing Time

Thoracic limbs had higher non-normalised **step widths** than pelvic limbs (0.13m versus 0.11m, respectively), but only twelve dogs returned values that were considered significantly different ($p<0.05$). Most participants had no significance between non-normalised thoracic and pelvic **stride length** values. There were minimal variations between mean stride length values for thoracic and pelvic limbs (0.74m (0.04) ± 0.15, and 0.74m (0.05) ± 0.16). Twenty-nine dogs had significantly different values for **swing time** between thoracic and pelvic values. Pelvic values were higher compared to forelimb values (0.22s (0.007) ± 0.02, and 0.19s (0.006) ± 0.03, respectively). PID 10 and 41 had no significant differences for swing time, whereas thoracic and pelvic values had minimal differences.

4.3.2.1.3 Velocity

The average speed for each dog across their 15 trials was combined to calculate an average speed for the non-mobility impaired group. The data, expressed as mean (standard error mean) and ± standard deviation for all 31 non-mobility impaired dogs are as follows: 1.67m/s (0.06) ± 0.31.

4.3.2.2 *Mobility impaired Dogs*

A Kruskal-Wallis or ANOVA test was also undertaken following the Shapiro-Wilk test for the 31 dogs in the mobility impaired group. The methodology remained the same as for non-mobility impaired dogs. However, the mobility impaired group was divided into 'combined' (where both thoracic and pelvic limbs were affected) 'thoracic limb' (where only thoracic limbs were affected) and pelvic limb (where only pelvic limbs were affected). The p-values ($p<0.05$) of these tests for corresponding comparisons for the 'combined', 'thoracic' and 'pelvic' groups are shown in Table 4.7. Again, LT/RT corresponds to left/right thoracic limb and LP/RP signifies left/right pelvic limb. The participant identification (PID) number is shown

on the left column to maintain dog anonymity. To the far left in Table 4.7, the sub-categories for mobility impairments can be found with the respective dogs who fit into these categories. All data within this subset is **non-normalised**.

4.3.2.2.1 *Cycle Time, Duty Factor and Stance Time*

All thirty-one dogs with mobility impairments had higher **cycle times** (s) in the thoracic limb compared to the pelvic limb. However, in all cases, this difference between individual thoracic and pelvic limb was found to be statistically insignificant. All thirty-one dogs had higher duty factors for thoracic limbs compared to pelvic limbs. The majority of dogs had p=<0.05 for LT versus LP, and RT versus RP limbs. Average values for LT and RT **duty factor** were 0.33s (0.01) ± 0.12, and 0.39s (0.01) ± 0.15, respectively. Similarly, LP and RP limb means were 0.37s (0.3) ± 0.14 and 0.34s (0.28) ± 0.16. PID 12 and PID 28 were the only participants with p<0.05 for duty factor. However, thoracic values (both LT and RT) were higher than LP and RP values. PID 27 and PID 29 were the only dogs with significant values for LP versus RP limbs. Participants showed differences between LT and LP, and RT and RP **stance time** values. However, differences between mean LT and RT values (0.14s and 0.15s), and LP and RP (0.13s and 0.13s) were relatively small. PID 12 was the only participant with a value of <0.05 for LT versus RT. Three dogs PID 24, PID 28 and PID 63 (all with pelvic mobility impairments) had values that were statistically significant, although thoracic values still remained higher than pelvic values.

4.3.2.2.2 *Step Width, Stride Length and Swing Time*

One dog (PID 24), had a **stride length** (m) that was deemed statistically significant <0.05 for LT versus LP (Table 4.7 and Figure 4.10). There was little-to-no variation between LT, RT, and LP and RP values (all equalled ~0.53m) for all 31 mobility impaired participants. Similarly, to non-mobility impaired dogs, swing time pelvic limb values (LP = 0.21m (0.004) ± 0.01, RP = 0.22m (0.007) ± 0.02) were higher than thoracic values (LT = 0.19m (0.003) ± 0.009, RT = 0.20m (0.004) ± 0.01). Many participants had significant differences between LT versus LP, and RT and RP **swing times**. One dog (PID 51) had significant differences between LT and RT limbs,

and PID 28 had a p-value of 0.004 between LP and RP. Thoracic limbs had higher **step widths** (expressed as metres (m)) than pelvic limbs (0.11m versus 0.08m, respectively).

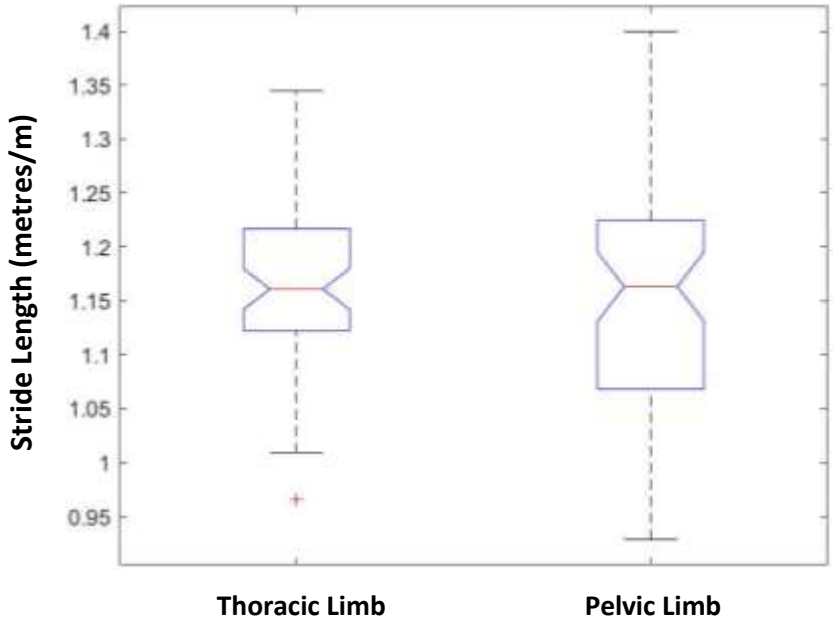

Figure 4. 10. Non-normalised stride length values for thoracic and pelvic limbs for participants 24. Median values are represented by the horizontal red line. Whiskers have end-points that correspond to minimum and maximum values for thoracic and pelvic values. Outliers are determined by red crosses.

4.3.2.2.3 *Velocity*

As before, t-tests and Wilcoxon rank-sum statistical tests were used in the comparison of left and right thoracic limbs, versus left and right pelvic limb values to determine statistical significance in the 31 mobility impaired dogs. Data are presented in Table 4.8.

		Cycle Time (p-values)				Duty Factor (p-values)				Stance Time (p-values)				Step Width (p-values)		Stride Length (p-values)				Swing Time (p-values)			
		LT vs RT	LT vs LP	RT vs RP	LP vs RP	LT vs RT	LT vs LP	RT vs RP	LP vs RP	LT vs RT	LT vs LP	RT vs RP	LP vs RP	Thoracic Limbs	Pelvic Limbs	LT vs RT	LT vs LP	RT vs RP	LP vs RP	LT vs RT	LT vs LP	RT vs RP	LP vs RP
	PID																						
Combined Limbs	1	0.999	0.999	0.957	0.917	0.232	***<0.0001***	***<0.0001***	0.728	0.826	***<0.0001***	***<0.0001***	0.663	0.634	0.784	0.999	0.717	0.918	0.986	0.330	***<0.0001***	***<0.0001***	0.910
	8	0.985	0.962	0.985	0.965	0.993	***<0.0001***	***<0.0001***	***0.013***	0.997	***<0.0001***	***<0.0001***	0.350	0.470	0.569	0.998	0.876	0.924	0.999	1	***<0.0001***	***<0.0001***	0.429
	9	0.891	0.804	0.950	0.889	0.508	0.474	***<0.0001***	0.455	0.977	0.673	0.091	0.841	0.150	***0.028***	0.992	0.991	0.992	0.994	0.435	0.883	***<0.0001***	0.177
	31	0.999	0.999	0.999	0.999	0.974	0.096	0.155	0.920	0.921	***0.035***	0.264	0.430	0.032	***0.026***	0.941	0.905	0.999	1	0.986	0.343	0.388	0.975
	34	0.620	0.421	0.479	0.616	0.896	***0.001***	0.073	0.958	0.999	***0.001***	0.091	0.667	0.226	0.529	0.875	0.278	0.991	0.866	0.436	0.105	0.998	0.905
	50	0.998	0.988	0.996	0.999	0.668	0.353	0.968	0.999	0.621	***0.020***	0.999	0.271	***0.001***	***0.002***	0.794	0.984	0.480	0.998	0.635	0.305	0.961	0.999
	54	0.876	0.383	0.375	0.883	0.492	0.035	***<0.0001***	0.663	1	***0.002***	***<0.0001***	0.752	0.181	***0.004***	0.998	0.337	0.501	0.999	0.113	0.712	***0.001***	0.993
Thoracic Limb	7	0.999	0.901	0.934	0.999	0.965	0.270	0.908	0.915	0.929	0.996	0.999	0.993	0.218	***0.002***	0.985	0.997	0.997	1	0.905	0.221	0.999	0.671
	32	0.979	0.999	0.998	0.989	0.963	***<0.0001***	***<0.0001***	0.999	0.968	***<0.0001***	***<0.0001***	1	0.588	0.575	0.990	0.973	0.997	0.999	0.996	***<0.0001***	***<0.0001***	0.990
	36	0.936	0.930	0.755	0.748	0.758	***0.002***	0.011	0.969	0.325	***0.002***	0.229	0.999	0.0179	0.631	0.826	0.980	0.999	0.942	0.573	***<0.0001***	0.056	0.921
	37	0.985	0.988	0.765	0.989	0.299	***<0.0001***	***<0.0001***	0.793	0.250	***<0.0001***	***<0.0001***	0.780	***<0.0001***	0.666	0.969	0.956	0.949	0.931	0.136	***<0.0001***	***<0.0001***	0.200
	46	0.997	0.997	0.999	0.999	0.112	***<0.0001***	***<0.0001***	0.949	0.999	***0.003***	0.115	0.552	0.611	***0.017***	0.923	0.998	0.953	0.993	0.142	***0.003***	***<0.0001***	0.997
	51	0.634	0.729	0.660	0.753	0.215	***<0.0001***	***<0.0001***	0.212	0.177	***<0.0001***	***0.001***	0.185	***0.026***	***0.036***	0.993	0.870	0.979	0.809	0.060	***<0.0001***	***0.0001***	0.277
	55	0.160	0.999	0.208	1	0.978	***<0.0001***	***0.031***	0.401	0.997	***<0.0001***	***0.028***	0.353	0.152	***<0.0001***	0.827	0.591	0.989	0.882	0.156	***<0.0001***	0.179	0.286
	57	0.987	0.098	0.726	0.820	1	***0.007***	***0.042***	0.969	0.909	0.667	0.189	0.988	0.873	0.590	0.965	0.487	0.564	0.979	0.866	***<0.0001***	***<0.0001***	0.874
Pelvic Limbs	5	0.751	0.635	0.415	0.307	0.998	***<0.0001***	***0.0006***	0.584	0.994	***<0.0001***	***<0.0001***	0.991	***0.001***	0.270	0.999	0.999	0.577	0.677	0.790	***<0.0001***	0.149	0.302
	12	0.999	1	0.794	0.753	***<0.0001***	***<0.0001***	***<0.0001***	0.061	***<0.0001***	***<0.0001***	***<0.0001***	0.243	0.157	0.792	0.995	0.857	0.998	0.976	***0.014***	***<0.0001***	***0.0001***	0.993
	24	0.934	0.291	0.719	0.998	0.439	***<0.0001***	0.349	0.085	0.167	***<0.0001***	0.484	***0.015***	0.990	0.202	0.522	***<0.0001***	0.937	0.064	0.845	0.999	0.628	0.977
	27	0.990	0.924	0.747	0.999	0.844	***<0.0001***	***<0.0001***	***0.0004***	0.755	***<0.0001***	***<0.0001***	0.383	***<0.0001***	***0.027***	0.947	0.999	0.779	0.950	0.966	***<0.0001***	***<0.0001***	0.425
	28	0.987	0.998	0.968	0.992	***0.008***	***<0.0001***	***<0.0001***	***0.001***	0.153	***0.016***	***0.0004***	***0.018***	0.190	0.347	0.950	0.983	0.974	0.992	0.309	***0.0001***	***<0.0001***	***0.004***
	29	0.879	0.992	0.997	0.914	0.946	***<0.0001***	***<0.0001***	0.306	0.892	***0.001***	***0.0007***	0.756	0.385	0.651	0.963	0.976	0.978	0.964	0.410	***0.0003***	***0.0003***	0.382
	39	0.792	0.987	0.975	0.998	0.801	0.172	***0.008***	0.997	0.908	***0.010***	***0.013***	0.798	***<0.0001***	***<0.0001***	1	0.980	0.223	0.442	0.895	0.571	0.082	0.985
	42	0.994	0.950	0.887	0.999	0.801	0.172	***0.008***	0.997	0.990	***<0.0001***	***<0.0001***	0.850	0.521	0.667	0.945	0.841	0.997	0.964	0.990	***<0.0001***	***<0.0001***	0.561
	43	0.969	0.958	0.994	0.990	0.970	***<0.0001***	***<0.0001***	0.949	0.996	***<0.0001***	***<0.0001***	0.997	0.618	0.116	1	0.999	0.999	0.996	0.999	***<0.0001***	***<0.0001***	0.645
	47	0.797	0.999	0.797	0.999	0.999	0.106	0.150	0.998	0.957	0.351	0.117	0.999	0.871	0.991	0.987	0.990	0.999	0.999	0.920	0.233	0.072	0.999
	49	0.999	0.973	0.998	0.964	0.651	0.064	0.086	0.572	0.919	0.075	0.252	0.613	0.141	0.991	0.852	0.998	0.982	0.991	0.309	0.355	***0.037***	0.914
	53	0.999	0.210	0.035	0.893	0.981	***<0.0001***	***0.024***	0.453	0.993	***<0.0001***	***0.007***	0.517	0.907	0.451	0.993	0.810	0.429	0.984	0.984	***0.020***	0.999	0.068
	63	0.997	0.920	0.915	0.998	0.957	***0.0002***	***<0.0001***	0.196	0.983	***<0.0001***	***<0.0001***	***0.049***	0.062	0.816	0.991	0.967	0.999	0.996	0.972	***<0.0001***	***<0.0001***	0.273
	64	0.999	0.363	0.548	0.987	0.954	0.061	0.352	0.512	0.975	***<0.0001***	0.109	0.074	***0.038***	0.125	0.994	0.732	0.570	1	0.775	***0.015***	0.239	0.162

Table 4. 17. A table to show the p-values for each spatiotemporal parameter for non-mobility impaired dogs. Significance level was set of $p<0.05$. p-values were obtained by either Analysis of variance (ANOVA) or Kruskal-Wallis statistical test. Statically significant values for non-mobility impaired dogs are highlighted in **bold** and *italics*. Definitions for the above spatiotemporal parameters can be found in the List of Abbreviations.

		Cycle Time (p-values)	Duty Factor (p-values)	Stance Time (p-values)	Step Width (p-values)	Stride Length (p-values)	Swing Time (p-values)
		Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs	Thoracic Limbs vs Pelvic Limbs
	PID						
Combined Limbs	**1**	0.750	***<0.0001***	***0.028***	***0.001***	***0.003***	***<0.0001***
	8	0.551	***<0.0001***	***<0.0001***	***<0.0001***	0.373	***<0.0001***
	9	0.797	***<0.0001***	***<0.0001***	***<0.0001***	0.695	***0.001***
	31	0.952	***<0.0001***	***<0.0001***	0.190	0.583	0.070
	34	***0.050***	***0.010***	***<0.0001***	***0.007***	0.141	***0.034***
	50	0.699	0.142	0.142	***0.012***	0.196	0.159
	54	***0.030***	***<0.0001***	***<0.0001***	***<0.0001***	***0.030***	***0.001***
Thoracic Limb	**7**	0.869	0.078	0.078	0.054	1	0.161
	32	0.772	***<0.0001***	***<0.0001***	0.827	0.657	***<0.0001***
	36	0.158	***<0.0001***	***<0.0001***	0.262	0.728	***<0.0001***
	37	0.969	***<0.0001***	***<0.0001***	0.140	0.990	***<0.0001***
	46	0.933	***<0.0001***	***<0.0001***	0.658	0.924	***<0.0001***
	51	0.202	***<0.0001***	***<0.0001***	***0.015***	0.784	***<0.0001***
	55	***0.018***	***<0.0001***	***<0.0001***	0.209	0.513	***<0.0001***
	57	0.366	***<0.0001***	***<0.0001***	***0.048***	0.057	***<0.0001***
Pelvic Limbs	**5**	0.775	***<0.0001***	***<0.0001***	***<0.0001***	0.340	***<0.0001***
	12	0.494	***<0.0001***	***<0.0001***	***<0.0001***	0.391	***<0.0001***
	24	***0.047***	***<0.0001***	***<0.0001***	***0.003***	0.405	***<0.0001***
	27	0.247	***<0.0001***	***<0.0001***	0.511	0.567	***<0.0001***
	28	0.587	***<0.0001***	***<0.0001***	0.525	0.975	***0.010***
	29	0.807	***<0.0001***	***<0.0001***	0.778	0.601	***<0.0001***
	39	0.955	***<0.0001***	***<0.0001***	0.427	0.104	***<0.0001***
	42	0.377	***<0.0001***	***<0.0001***	0.222	0.661	***0.003***
	43	0.563	***<0.0001***	***<0.0001***	***<0.0001***	0.953	***0.003***
	47	0.560	***0.002***	***0.002***	***<0.0001***	0.891	***0.029***
	49	0.856	***0.001***	***0.001***	0.482	0.891	***<0.0001***
	53	***0.001***	***<0.0001***	***<0.0001***	***<0.0001***	0.088	***<0.0001***
	63	0.347	***<0.0001***	***<0.0001***	***0.007***	0.766	***<0.0001***
	64	***0.036***	***0.003***	***0.003***	0.570	0.101	***0.001***

Table 4. 18. p-values shown for each spatiotemporal parameter for n=31 mobility impaired dogs when individual thoracic and pelvic values were combined. Significance level was set of $p<0.05$. p-values were obtained by either T-test or Wilcoxon Rank-Sum statistical test. Statistically significant values for non-mobility impaired dogs are highlighted in **bold** and *italics*.

4.3.2.2.4 *Cycle Time, Duty Factor and Stance Time*

Twenty-five participants showed no statistical difference between thoracic and pelvic **cycle times**. There were differences on thoracic and pelvic cycle times (0.34s (0.01) ± 0.02, and 0.23s (0.01) ± 0.02, respectively), due to differences in body size. Twenty-nine dogs had significant differences between thoracic and pelvic **duty factors**; thoracic values were generally higher than pelvic values (0.42s (0.01) ± 0.02, compared to 0.37s (0.01) ± 0.03). Two dogs (PID 7 and PID 50) yielded results greater than 0.05. Twenty-nine dogs had statistically significant differences between thoracic and pelvic **stance time** values, where thoracic limb values were higher than pelvic limb values (0.14s (0.007) ± 0.01) and 0.13s (0.006) ± 0.01). Again, PID 7 and PID 50 had values which were not statistically significant for stance time.

4.3.2.2.5 *Step Width, Stride Length and Swing Time*

Thoracic limbs had higher **step widths** than pelvic limbs (0.12m versus 0.10m, respectively). Fourteen dogs returned values which were significant ($p<0.05$). Most participants had no significant difference between thoracic and pelvic stride length values. There were very minimal variations between mean **stride length** values for thoracic and pelvic limbs (0.53m (0.01) ± 0.04, and 0.53m (0.01) ± 0.03). Twenty-eight dogs had significant values for **swing time** between thoracic and pelvic values. Similarly, to non-mobility impaired dogs, pelvic values were higher compared to thoracic values (0.19s (0.008) ± 0.009, and 0.21s (0.008) ± 0.02, respectively). PID 7, 31 and 50 had no significant differences for swing time, where thoracic and pelvic values had minimal variation.

4.3.2.2.6 *Velocity*

The average speed for each dog across their 15 trials was combined to calculate an average speed for each mobility category to give an overall non-normalised speed in m/s for dogs with combined, thoracic and pelvic mobility impairments. The data, expressed as mean (standard error mean) and ± standard deviation for all 31 mobility impaired dogs are as follows: (1)

combined 1.55m/s (0.09) ± 0.24, (2) thoracic limbs 1.83m/s (0.08) ± 0.21, (3) pelvic limbs 1.55m/s (0.08) ± 0.33.

4.3.3 *Between-Participant Kinetic (Force Plate) Data*

4.3.3.1 *Kinetic (Force Plate) Data*

The **normalised** PVF data were compared between both groups of mobility impaired dogs (n=31) and non-mobility impaired dogs (n=31). The data were non-normally distributed; therefore, a Kruskal-Wallis test was used to determine statistically significant differences between the datasets set at an alpha of $p \leq 0.05$.

There were differences between normalised mean thoracic PVF (expressed as Newtons per Kilogram (N/Kg)) values between non-mobility impaired dogs and mobility impaired dogs. Mobility impaired dogs generally had higher thoracic PVF values than non-mobility impaired dogs (2.486N/Kg (0.20) ± 0.67 and 1.249N/Kg (0.13) ± 0.718, respectively). Dogs with pelvic limb mobility impairments had the highest thoracic PVF values, compared non-mobility impaired dogs. However, those with thoracic and combined mobility impairments do not (Figure 4.6). Data was subcategorised into PVF means for thoracic limbs, total PVF for pelvic limbs and ratios of PVF thoracic and pelvic ratios for mobility impaired versus non-mobility impaired dogs. The Kruskal-Wallis output for PVF means for thoracic limbs was $H(9.8) = 3$, $p = .021$, PVF for pelvic limbs was $H(13.8) = 3$, $p = 0.003$. The Kruskal-Wallis results for thoracic to pelvic limb PVF ratio was $H(4.7) = 3$, $p = 0.019$.

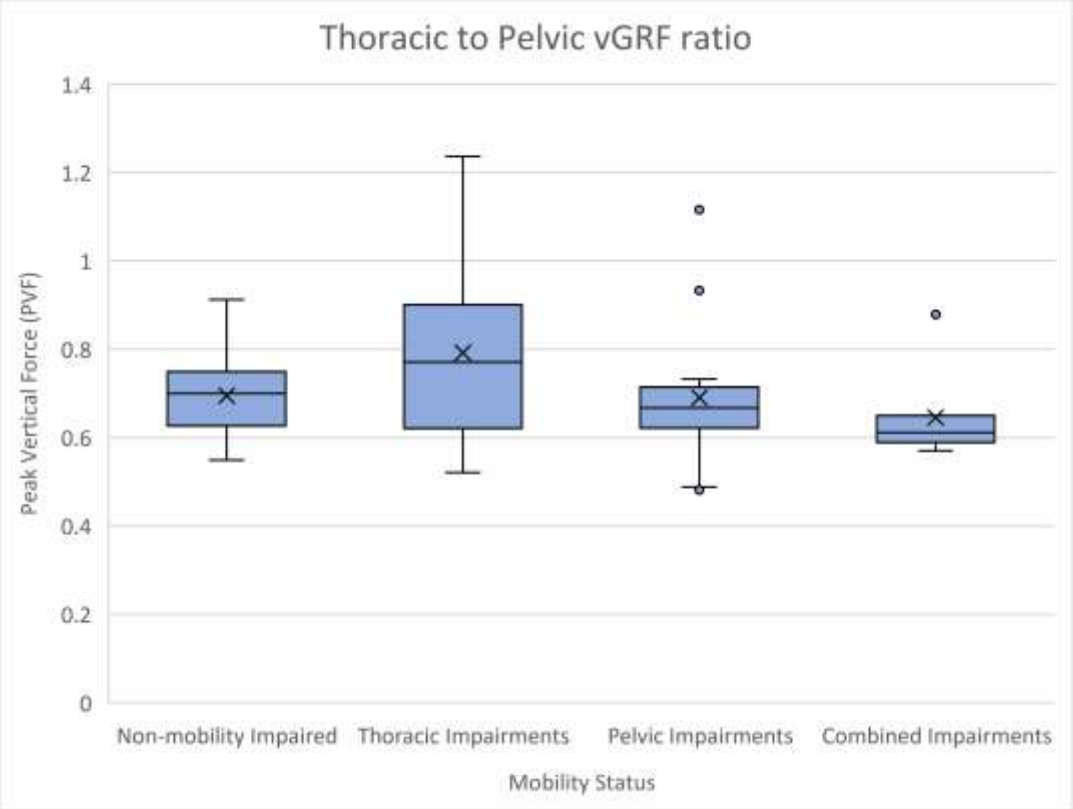

Figure 4. 11. A boxplot to demonstrate the ratio of pelvic and thoracic PVF means across all mobility and non-mobility impairment categories. Each category is represented by PVF (normalised by body mass). 'X' represents the median, the whiskers (vertical lines) extend from the ends of the box to show the minimum value and maximum value, black lines represent the mean values. Outliers are highlighted by solid blue dots.

Furthermore, when all mobility impaired dogs were treated as a single group, they had higher thoracic PVFs (2.486N/Kg (0.20) ± 0.67 and 1.249N/Kg (0.13) ± 0.718, respectively). However, when separated, those with thoracic issues (0.866N/Kg (0.09) ± 0.28) have lower PVF compared to those with combined mobility issues (1.090N/Kg (0.09) ± 0.22). Those with pelvic issues (1.237N/Kg (0.08) ± 0.28) had the highest PVF out of all the mobility impaired cohorts.

Mean pelvic PVF were higher in non-mobility impaired dogs (0.862N/Kg (0.09) ± 0.54) compared to mobility impaired dogs (0.733N/Kg (0.04) ± 0.15). However, dogs with pelvic limb mobility impairments yielded the highest mean pelvic PVF in comparison with those who had thoracic and combined mobility impairments.

	Mean (standard error of the mean) ± standard deviation		
	Total Thoracic vGRF mean	Total Pelvic vGRF mean	Total Thoracic to Pelvic vGRF ratio
Non-mobility impaired	1.249 (0.13) ± 0.71	0.862 (0.09) ± 0.54	0.695 (0.01) ± 0.09
Mobility impaired:			
- Thoracic Limb	0.886 (0.09) ± 0.28	0.653 (0.04) ± 0.12	0.791 (0.08) ± 0.22
- Pelvic Limb	1.237 (0.08) ± 0.32	0.855 (0.05) ± 0.22	0.706 (0.04) ± 0.15
- Combined Limbs	1.090 (0.09) ± 0.22	0.691 (0.04) ± 0.11	0.646 (0.04) ± 0.11

Table 4. 19. The mean PVF (expressed as Newtons (N)), standard error of mean and standard deviation from the PVF for thoracic and pelvic limbs are shown in this table. Non-mobility impaired and mobility impaired dogs and their subcategories (thoracic/pelvic/combined) are included.

Mobility impaired participants had higher thoracic to pelvic PVF ratios (0.714N/Kg (0.05) ± 0.16), compared to non-mobility impaired participants (0.695N/Kg (0.02) ± 0.09), meaning that PVFs were relatively higher in the thoracic limb in these dogs. Thoracic limb mobility impairments returned the highest ratios compared to dogs who had pelvic limb and combined limb issues

4.3.4 *Between-Participant Kinematic (Spatiotemporal) Data*

4.3.4.1 *Kinematic (Spatiotemporal) Data*

All **normalised** spatiotemporal data was analysed using the one-way ANOVA statistical test as data was normally distributed, as described above in Section 2.8.3.3, the normalised spatiotemporal results are considered dimensionless and thus, have no units. The majority of spatiotemporal parameters returned no statistically significant difference between mobility and non-mobility impaired groups (Table 4.10). Total thoracic and pelvic means for **cycle time** and **step width** were the only parameters to yield a statistically significant result ($p \leq 0.05$).

	p values as determined by one-way ANOVA		
	Total Thoracic mean	Total Pelvic mean	Total Thoracic to Pelvic ratio
Cycle time	0.692	0.773	0.05
Duty factor	0.817	0.713	0.826
Stance time	0.771	0.535	0.814
Step width	0.876	0.203	0.046
Stride length	0.895	0.925	0.120
Swing time	0.356	0.578	0.564

Table 4. 20. This table shows all six spatiotemporal parameters (cycle time, duty factor, stance time, step width, stride length and swing time) that were analysed for both the non-mobility and mobility impaired cohort. A one-way ANOVA was used to analyse thoracic and pelvic means, alongside thoracic and pelvic ratios. Statistical significance was set to $p \leq 0.05$.

4.3.4.1.1 *Cycle Time, Duty Factor and Stance Time*

There were minimal variations for **cycle time** between thoracic and pelvic values within the mobility impaired and non-mobility impaired groups. The pelvic mobility impaired group was the only sub-category not to have a thoracic to pelvic ratio of 1.0 for cycle time, as the result was 0.989. Similarly, to cycle time, there was also minimal disparity in **duty factor** values. However, thoracic limbs always had higher mean values when compared to pelvic limbs. Non-mobility

impaired participants had higher duty factors (0.471 (0.007) ± 0.04) compared to mobility impaired participants (0.464 (0.01) ± 0.03), but differences were not statistically significant. Dogs with thoracic mobility impairments had a higher thoracic to pelvic limb ratio compared to non-mobility, pelvic and combined impairment categories. Thus, non-mobility impaired dogs had their pads on the ground for a higher percentage of the gait cycle than mobility impaired dogs. Similarly, in Figure 4.17, all duty factor values (apart from 1-2 outliers), are below 0.5, indicating that all dogs were in a trotting phase, as opposed to walking. Mobility impaired participants had a marginally higher **stance time** compared to non-mobility impaired dogs (0.214 compared to 0.212, respectively). Pelvic mobility impaired dogs had the lowest stance time (0.205 (0.01) ± 0.04). Furthermore, this category also had the lowest thoracic to pelvic limb ratio. The combined mobility impairment category had the highest stance time (0.222 (0.01) ± 0.03) for their body size, compared to non-mobility impaired dogs. Supporting the hypothesis that mobility impaired dogs were moving slower compared to non-mobility impaired dogs.

4.3.4.1.2 *Step Width, Stride Length and Swing Time*

Dogs with no known mobility impairments and those with detected thoracic impairments had very similar normalised **step widths** (0.148 and 0.147, respectively). Participants who were identified to have pelvic limb mobility impairments were found to have the smallest step width (0.134 (0.009) ± 0.03). Consequently, dogs with mobility impairments had smaller step widths than those without mobility impairments (0.140 (0.01) ± 0.02, compared to 0.148 (0.007) ± 0.04) but were not considered statistically significant.

Almost all dogs, except those in the combined mobility impairment category (0.989) had thoracic to pelvic ratios of 1.0 for normalised **stride length**. Moreover, this category also had the lowest stride length value (0.802 (0.07) ± 0.16) compared to the non-mobility impaired category (0.857 (0.03) ± 0.2). Subsequently, the non-mobility impaired category had higher stride lengths (0.857) than the mobility impaired group (0.821). Hence, non-mobility impaired dogs were moving more slowly in comparison. As discussed above, there were minimal variations between the cycle time

of mobility impaired dogs and non-mobility impaired dogs, however, non-mobility impaired dogs were found to have higher average speed (as discussed below), supporting the statement that they were generally moving faster than mobility impaired dogs.

The overall normalised **swing time** was the lowest for non-mobility impaired participants (0.232 (0.005) ± 0.03) compared to the mobility impaired dogs (0.246 (0.007) ± 0.03). The combined mobility impairment group had the longest swing time (0.255), with the pelvic limb category having the lowest swing time (0.237) from the mobility impaired cohort. Hence, mobility impaired dogs did not walk as fast, and thus, had shorter gait cycles compared to non-mobility impaired dogs. Boxplots with the upper and lower quartiles for all the kinematic parameters can be found in Figure 4.8 below with all the mobility (sub)categories on the x-axis.

4.3.4.1.3 *Velocity*

A one-way ANOVA found statistically significant differences between the **average speed** between mobility and non-mobility impaired groups (p= 0.048). Speed was normalised by body mass and an additional Froude number was calculated. Non-mobility impaired groups were found to have a higher average speed compared to mobility impaired dogs (1.66 m/s/Kg, compared to 1.62 m/s/Kg). The combined mobility impairment group had the slowest average speed in contrast to thoracic and pelvic impairments (1.56 m/s/Kg, compared with 1.64 m/s/Kg and 1.62 m/s/Kg, respectively). A mean Froude number (Fr) was also calculated for dogs who had thoracic and pelvic mobility impairments. However, there was not a statistically significant difference (p=0.45). Dogs with thoracic mobility impairments were found to have a higher Froude number than dogs with pelvic mobility impairments (0.76 compared to 0.66).

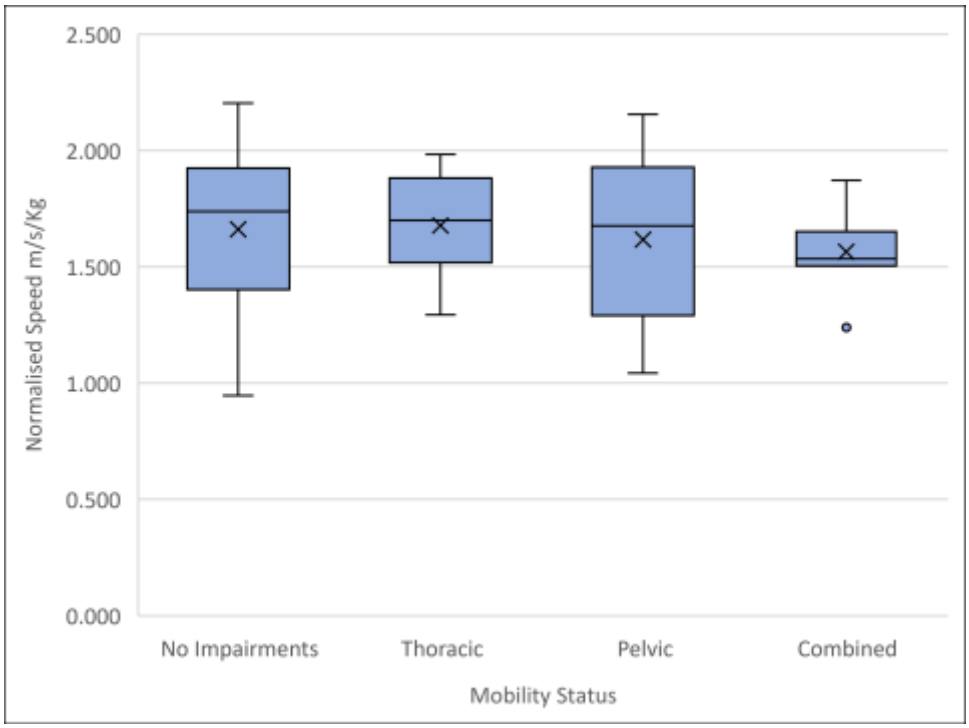

Figure 4. 12. Boxplots to demonstrate average speed across non-mobility impaired dogs and mobility impaired cohorts during a total of 25 trotting trials. 'X' represents the median, the whiskers (vertical lines) extend from the ends of the box to show the minimum value and maximum value. Mean values are shown via the black lines. Outliers are highlighted by solid dots.

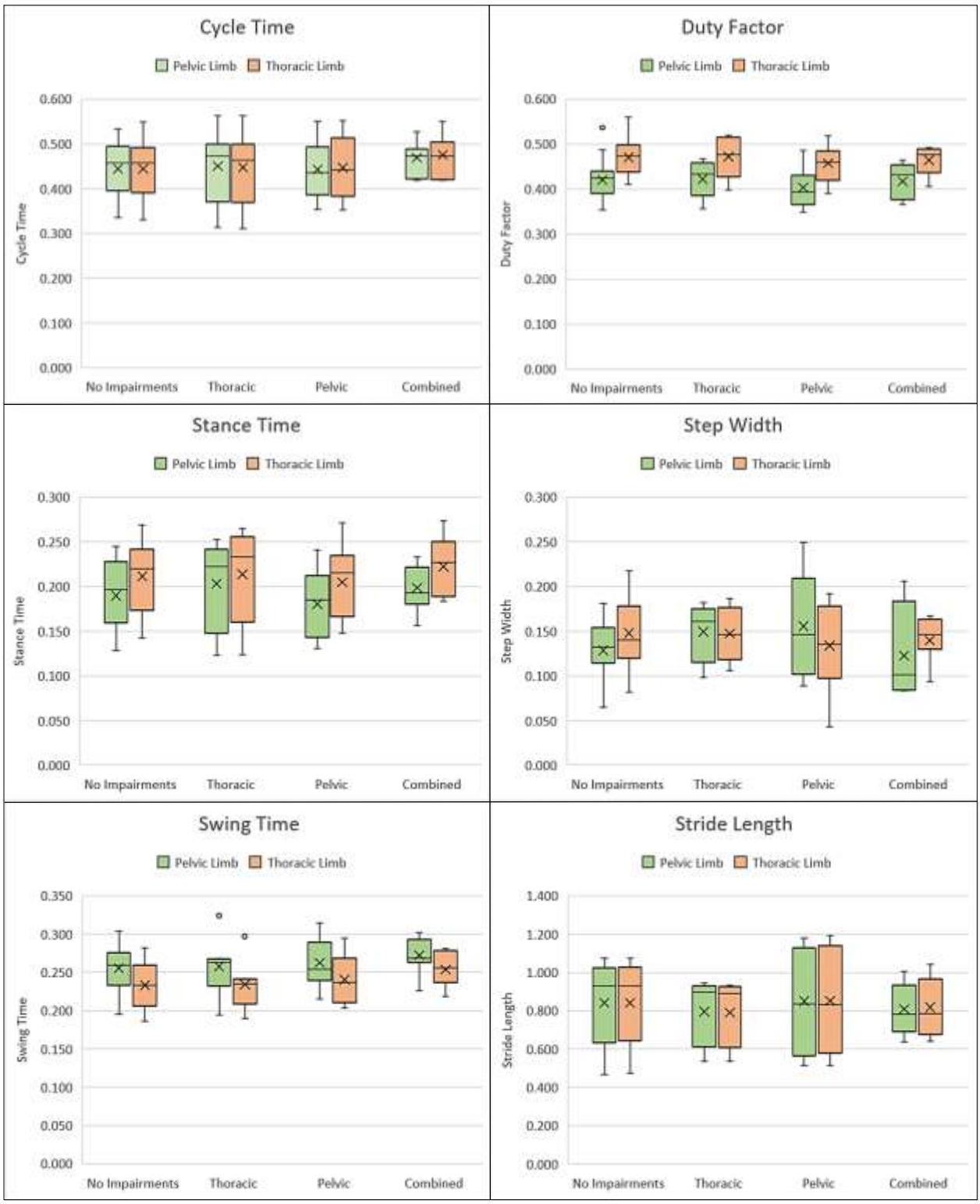

Figure. 4.13. Boxplots to demonstrate all six spatiotemporal parameters (cycle time, duty factor, stance time, step width, stride length and swing time). 'X' represents the mean, the whiskers (vertical lines) extend from both ends of the boxplot to show the minimum value and maximum value. Outliers are highlighted by solid dots.

4.4 Discussion

Several veterinary studies have used kinetic gait data to determine mobility status and questionnaire validation (Walton et al., 2013; Hercock et al., 2009; Kim et al., 2011; Besancon et al., 2004; Jevens et al., 1993). However, this project combined kinetic and kinematic canine gait data to assess not only PVF but also various spatiotemporal parameters which are sensitive to subtle changes in mobility, enabling the validation of GenPup-M.

Consequently, large volumes of data were retrieved for each participant relating to PVF, cycle time, duty factor, stance time, step width, stride length and swing time (Figure 4.8, Table 4.6; 4.7; 4.8; 4.9). There were initial plans to include data additional kinematic gait data relating to joint angles, however, this had to be aborted due to a relatively short time frame for the project.

4.4.1 Kinetic Data for Non-Mobility Impaired Dogs

There is a consensus among veterinary professionals that dogs unevenly distribute their weight between thoracic limbs and pelvic limbs in terms of PVF (60:40, respectively ((Olson and Carithers, 1982)). Non-mobility impaired dogs in this study followed this approximation closely, as there were noticeably higher PVF for thoracic limbs compared to PVF for pelvic limbs (Figure 4.6). Although, likely due to the large variation between dog breeds, there were large within-participant variation in relative thoracic and pelvic PVF (Figure 4.5). There seemed to be equal distribution of weight between left and right thoracic and pelvic limbs in non-mobility impaired dogs. PID 19 was the only dog that recovered a statistically significant result between the left and right thoracic limbs. However, PID was a 5-month-old West Highland White Terrier (WHWT) who became very excited when crossing the force plate platforms. This excitement caused her to hop and jump during some trials, which may be the cause of this discrepancy, however, this dog was not discarded from the study as it was felt the trials retained did not contain giddiness.

PID 41, 59 and 65 were the only dogs to yield non-statistically significant results for left thoracic limb versus left pelvic limb, and right thoracic limb versus right pelvic limb peak vertical force

(PVF). There were no known mobility impairments reported by the owner or identified by using the validated clinical examination sheet. However, the PVF for these dogs' results show they are more evenly weight-bearing across all four limbs, compared to other non-mobility dogs in this cohort. Interestingly, PID 41, 59 and 65 were either Labrador retrievers or Labradoodles, breeds commonly known for mobility impairments (McGreevy et al., 2018). The above PVF data could potentially show very early onset mobility impairments within these dogs. Perhaps a graphical representation of this data variation in thoracic to pelvic PVF in Labradors relative to the rest of the dataset would be interesting to scope the variation between breeds.

4.4.2 Kinetic Data for Mobility Impaired Dogs

Twenty-four (out of 31) mobility impaired dogs showed statistically significant differences between left thoracic versus left pelvic limb, and right thoracic right versus pelvic limb (all of which had higher thoracic PVF values compared to pelvic PVF values) ($p=<0.05$). The other seven mobility impaired dogs had minimal or equal PVF when compared. Three of these dogs had "combined" mobility impairments where they had impaired mobility in both thoracic and pelvic limbs (either unilaterally or bilaterally). PID 7 and PID 50 were among the dogs with the most severe mobility impairment dogs, with bilateral pelvic limb mobility impairments and unilateral thoracic limb impairments. Both of these dogs had minimal deviations between mean left thoracic and left pelvic PVF and right thoracic and right pelvic PVF. These data showed that these dogs were not distributing their weight according to the expected 60:40 ratio. However, both of these dogs were walked by their owner throughout the investigation. Jevens et al (1993) found that handlers can negatively impact the data by up to 7%, thus, it is possible the owner could have skewed the PVF slightly. In all cases (regardless of mobility impairment), all dogs had a higher a PVF on thoracic limbs compared to pelvic limbs.

4.4.3 Comparison of Kinetic Data for Non-Mobility and Mobility Impaired Dogs

When the mobility impaired cohort were compared to the non-mobility impaired cohort there were differences between mean thoracic PVF values. Consequently, mobility impaired participants had higher thoracic to pelvic PVF ratios compared to non-mobility impaired participants. This is due to more of the mobility impaired cohort having solely pelvic limb mobility impairments, and thus, weight-bearing more on their thoracic limbs. This statement is further supported as dogs with pelvic limb mobility impairments had the highest thoracic PVF values (Figure 4.8), compared to those with thoracic and combined mobility impairments. Fischer et al (2013) found a similar result when examining PVF in the ipsilateral and contralateral limbs in eight Beagles.

4.4.4 Comparison of Kinematic Data for Non-Mobility and Mobility Impaired Dogs

There was no statistically significant difference in the **cycle times** of individual limbs in the non-mobility impaired dogs. Cycle time is defined by the time interval between two successive occurrences of one of the repetitive events of locomotion; for example, the time between the same gait event (e.g. initial foot contact) happening again. For the within-dog analysis, the mobility impaired dogs had higher cycle times in the thoracic limb compared to the pelvic limb. When undertaking the between-dog analysis, there were minimal differences in cycle time between thoracic and pelvic values within the whole mobility impaired and non-mobility impaired groups. However, there were differences observed for stance time and swing time between the two cohorts which could be been caused by the presence of mobility impairments, but may have not directly affected the cycle time as a whole. Additionally, the amalgamation of data may have skewed these differences to be inconsequential for the between analysis

Duty factor is the fraction of the stride duration over which a foot remains on the ground and changes with speed. Duty factor was generally higher for thoracic limbs, compared to pelvic limbs

in the non-mobility impaired group. Mobility impaired dogs had higher duty factors compared to non-mobility impaired dogs. Duty factors of <0.5 suggest the dog was utilising a trotting gait, compared to a walking gait (>0.5) and the handlers had minimal effect on the speed that the dog was walking (a concern of Jevens et al., 1993). **Stance time** values were lower for mobility impaired dogs, supporting the above statement that mobility impaired dogs were moving slower than non-mobility impaired dogs. The stance time represents around 60% of the whole cycle time, it is representative of the time the foot is spent in contact with the ground. Thus, it is unsurprising that stance time is lower in the mobility impaired group and thus, they are walking at a slower speed, compared to non-mobility impaired dogs. Dogs with pelvic mobility impairments had the lowest stance time, potentially due to the pelvic limbs being primarily involved in propulsion during a canine gait event. Initially, there were concerns about the variation in **speed** drastically impacting the spatiotemporal findings in the present study. Again, speed further evidences that mobility impaired dogs walked slower in both absolute (m/s) and relative (Froude number) speed than non-mobility impaired dogs. This can be determined as a positive finding, as the variations in spatiotemporal results are not consequential of speed variation. Hence, gait and mobility are causing the differences seen between the mobility impaired and non-mobility impaired categories.

Step width is the distance measured between the line of progression of the left foot and the line of progression of the right foot (Fihl and Moeslund, 2008). Dogs with no known mobility impairments and those with detected thoracic impairments had very similar step widths. Participants who were identified to have pelvic limb mobility impairments were found to have the smallest step width. Consequently, dogs with mobility impairments had smaller step widths than those without mobility impairments. A wider step width requires more muscle activity. It is considered less economical than a narrower step width (Brach et al., 2005). Suggesting, that if muscle mass is reduced, muscle function is impaired, or the animal wants to actively reduce muscle force (e.g. to reduce pain), then this might lead to reduced step width.

There were minimal variations between mean **stride length** values for thoracic and pelvic limbs for mobility and non-mobility impaired dogs during the within-dog analysis. Interestingly, there were minimal variations between stride lengths for the between-cohort analysis. Although, the

combined mobility impairment category had the lowest stride length value, compared to the non-mobility impaired category. Potentially, this was due to multiple limbs being affected and the animal was less able to compensate on either thoracic or pelvic limbs, thus reducing its stride length.

Swing phase begins when the foot first leaves the ground and ends when the same foot touches the ground again. The swing phase makes up the other 40% of the gait cycle (Kano et al., 2016). The overall mean swing time was the lowest for non-mobility impaired participants compared to the mobility impaired dogs during the between-dog analysis. Once again supporting that mobility impaired dogs had longer gait cycles (as previously suggested by longer stance times and lower duty factor)

Throughout this study, when smaller breeds were compared against their medium and larger counterparts, it was found that most of the normalised spatiotemporal parameters (cycle time, duty factors, stance time and swing time) were lower for smaller breeds. All non-mobility impaired dogs recovered similar values, suggesting the qualitatively, smaller and larger dog breeds walk the same. Kim et al (2011) also found this when comparing kinetic and spatiotemporal variables in small and larger breed dogs. However, since the current study had a very small number of dogs <10kg (n=7), it is unlikely to have impacted the findings.

Waxman et al., 2008, stated that force plate analysis was the gold standard approach for quantifying mobility problems. However, there were some limitations of gait analysis which arose during this project, particularly when relating the gait data to clinical examination data (which was used to categorise dogs as mobility impaired or non-mobility impaired). For example, there is no one universal method for detecting pain or determining how animals will respond to a mobility impairment. As a result, every animal will not change their gait in exactly the same way when compensating for a mobility issue. Clinical examination can perhaps be a more sensitive way of identifying pain responses to extension or manipulation of a limb, or when observing transitional movements. Furthermore, for this study, joint kinematics were not investigated due to a restricted time frame. Therefore, without using clinical examination to assign dogs into mobility categories, in this study, information relating to the range of motion and pain when

manipulating specific joints would have been missed, potentially resulting in subtler orthopaedic conditions going unnoticed during quantitative gait analysis, thus, resulting in dogs being assigned into the wrong categories. Another limitation is perhaps inter-breed variation; there was a large cohort of various breeds for this study, hence, without a large sample size of all breeds, the gait analysis cannot fully account for these variations in the gait analysis. Whereas, clinical examination is less breed-specific and can be applied to all breeds, regardless of body weight. Similarly, the clinical examination contained a checklist which was completed for every dog, this ensured consistency when scoring and assigning mobility categories. However, it cannot be predicted how a dog will respond to retro-reflective markers attached to its fur, or when crossing floor-mounted force plates in a controlled manner. Consequently, this can cause errors in data collection and cause data to become skewed.

4.5 Conclusion

In the current study, kinetic and kinematic gait analysis was able to highlight the subtle differences between mobility impaired and non-mobility impaired cohorts. PIDs 41, 59 and 65 represent breeds that are particularly predisposed to developing mobility impairments, some values for these dogs were abnormal throughout the gait analysis when compared to other "normal" values. Although these dogs were considered non-mobility impaired, perhaps, gait analysis could have detected subtle deviations resulting in early-onset mobility impairments, or perhaps this could have been an error in data collection, for publication of this work, more investigation is required to assess where these deviations arose and what significance they carry. Clinical examination scores were used to categorise dogs as either mobility impaired or non-mobility impaired due to the extensive time frame required to create MATLAB code for gait analysis. However, the above results highlight the potential benefits of using clinical examination compared to gait analysis. Hercock et al., 2009 and Waxman, 2008 both state that gait analysis should be used, where possible, to support the visual gait assessment and clinical examination. Although further research is required to determine the correlation between the two forms of

objective gait assessment to ensure dogs participating in similar projects as the current study are coded as accurately as possible.

CHAPTER FIVE | VALIDATION OF GENPUP-M USING VETERINARY CLINICIAL EXAMINATION AND GAIT ANALYSIS

5.1 Background

To validate a questionnaire tool, it should be understood by all participants to answer correctly (Jain, Dubey and Jain, 2016). Hence, validity refers to the instrument's ability to accurately measure what it claims to. There are four different concepts of validity: (1) Content, (2) Face, (3) Construct, and (4) Criterion (Connell et al., 2018; Mousazadeh et al., 2017).

Face validity is judged by subjective assessment and is determined by review, not statistics. Face validity ensures that all questions are logical and relevant to the subject (Mousazadeh et al., 2017). Construct validity ensures a scale is valid and exhibits good psychometric properties to ensure it measures what it is intended to measure (Haynes et al., 1995) and evaluates the degree to which the instruments reflects the concept to be measured. Criterion validity is an estimate of the extent to which a questionnaire agrees with the gold standard format. Content validity should envelop more concepts of the questionnaire, it ensures the composition of the questionnaire is adequate for what it intends to measure (Frost et al., 2007).

In this study, GenPup-M will be validated using quantitative canine gait analysis, a validated veterinary clinical examination sheet (Harris et al., 2018) and a previously validated owner-reported canine mobility questionnaire (LOAD, Walton et al., 2013). The clinical examination sheet will be used as the gold-standard assessment of canine mobility impairments.

5.2 Materials and Methods

5.2.1 *Content Validity*

Previous literature and CMIs were reviewed to assess all relevant concepts prior to designing GenPup-M to ensure it covered all relevant parts of the subject (mobility impairments) it aimed

to measure. GenPup-M contained a total of twenty-four questions for owners to complete, including questions relating to supplements, terrain, frequency of walks, duration of walks and restriction of exercise, among others, to test the construct. Furthermore, GenPup-M contained ten scale/rank questions about the dog's mobility; with each question requiring the owner to provide a rating from 0-10 on the scale. This content is thought to assess many aspects which may impact canine mobility and account for environmental and dietary factors also.

5.2.2 *Face Validity*

Face validity was achieved by an orthopaedic specialist (EC) and a veterinary epidemiologist (Dr. Lauren Harris BSc PhD) reviewing the GenPup-M questionnaire. GenPup-M questionnaire has also been used as a part of the longitudinal 'Generation Pup' study, whereby dog owners complete questions relating to their dog's health and welfare. EC, LH and JM all review the online GenPup-M questionnaire to ensure JISC.co.uk resulted in an online version that was as similar as possible to the formatting used in GenPup for the Generation Pup longitudinal study.

5.2.3 *Construct Validity*

Construct validity was assessed by comparing the GenPup-M questionnaire responses using a validated veterinary clinical examination sheet (Harris et al., 2018). All dogs underwent a veterinary clinical examination by a qualified veterinary surgeon (NC/EC), which included visual gait assessment prior to canine gait analysis, this data was then used to categorise dogs as mobility impaired and non-mobility impaired. Using previous methodology (Hercock et al., 2009; Muller et al., 2016; Walton et al., 2013) the new GenPup-M questionnaire was also correlated with kinetic gait analysis (Peak Vertical Force (PVF)) to aid construct validity. The ratio between thoracic and pelvic PVF was used to assess the correlation between GenPup-M responses and quantitative gait analysis.

5.2.4 *Criterion Validity*

For criterion validity, GenPup-M was compared against a previously validated canine mobility scoring tool, the Liverpool Osteoarthritis in Dogs (LOAD) questionnaire, which was selected due to its frequent use in veterinary mobility research (Walton et al., 2013; Chiu et al., 2020; Roberts et al., 2021). Criterion validity indicates how well a newly constructed instrument correlates with a standardised, external measure of the disease. The LOAD questionnaire was replicated in an online version using JISC.co.uk to comply with COVID-19 restrictions. Owners were asked to complete the LOAD questionnaire after GenPup-M to reduce respondent bias for GenPup-M, and were made aware of any repetitive elements between both questionnaires. Further rationale for asking owners to complete the GenPup-M questionnaire first can be found in Section 2.2.4. EC and NC were blinded to all GenPup-M and LOAD responses until after clinical examination and gait analysis had been completed.

5.3 Results

5.3.1 *Comparison of GenPup-M and LOAD Questionnaire Responses*

The GenPup-M and LOAD questionnaire responses were correlated against one another using Spearman's rank correlation. The significance level was set at $p \leq 0.05$ (two-tailed). Total GenPup-M and LOAD scores were calculated using all of the questions relating to mobility within each questionnaire. There was a significant, positive relationship between total GenPup-M and LOAD scores. Spearman's correlation coefficient between GenPup-M and LOAD was $r^2 = 0.685$ ($p<0.001$) showing there is a significant moderate correlation for the inter-instrument comparisons (> 0.68 – 1 = a strong correlation (Taylor, 1990)). A scatterplot of GenPup-M versus LOAD is shown below (Figure 5.1). Kendall's tau_b was also used to investigate concordance and discordance more conservatively, as Kendall's tau is generally smaller when detecting a gross

error of sensitivity. Kendall's tau_b = 0.528 ($p<0.001$), showing there is still a significant relationship.

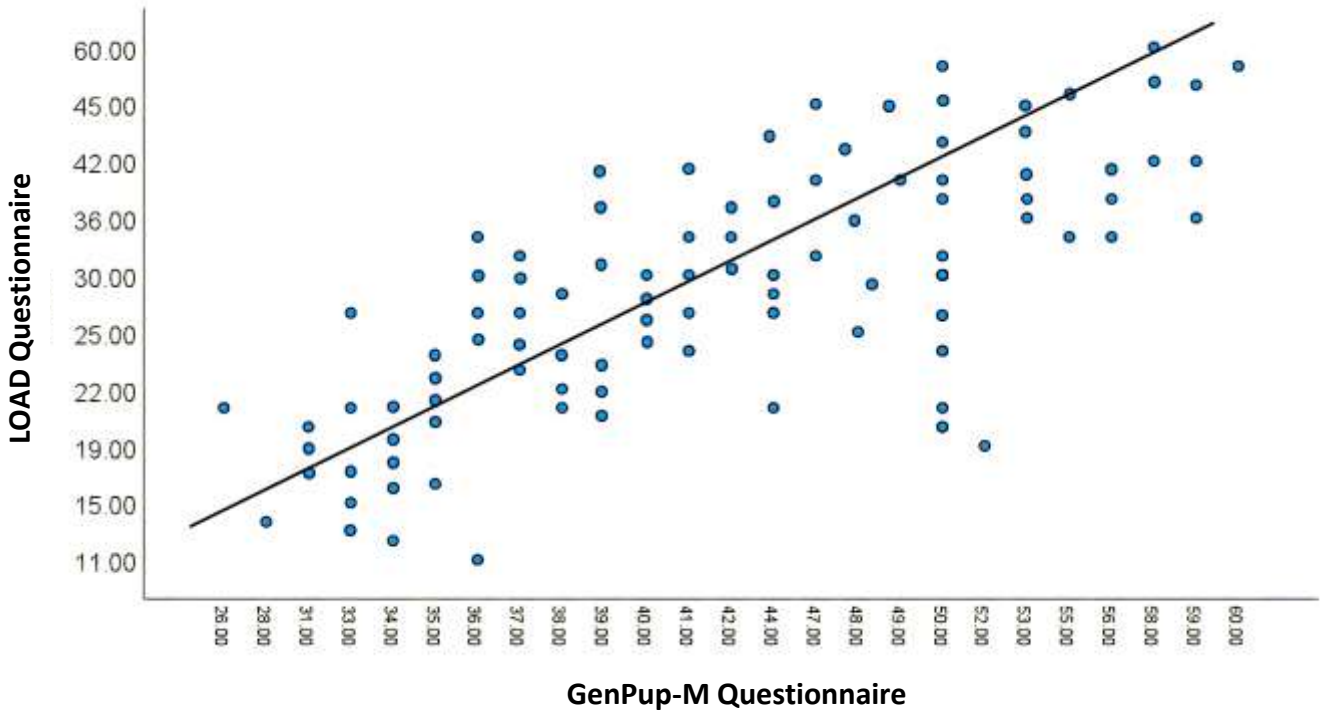

Figure 5. 2. Scatterplot of GenPup-M versus Liverpool Osetoarthritis in Dogs (LOAD) questionnaire scores. There was a significant, moderate positive correlation typical of inter-instrument comparisons. Spearman's Rank Correlation Coefficient = 0.69 ($p<0.001$). A black line of best fit is shown through the scatterplot.

5.3.2 *Veterinary Clinical Examination*

Spearman's rank correlation coefficient was used to determine if the veterinary total clinical examination scores and GenPup-M responses correlated with each other, and therefore, determine if owners' perceptions were in line with the veterinary judgement. There was a significant positive correlation between total GenPup-M questionnaire scores and clinical examination scores. The significance level was set at $p \leq 0.05$ (two-tailed). Spearman's correlation coefficient between GenPup-M and veterinary clinical examination was $r^2 = 0.742$ ($p<0.001$)

showing there is a significant strong, positive correlation between GenPup-M and veterinary clinical examination data (r^2 = > 0.68 – 1 = a strong correlation (Taylor, 1990)). Hence, higher GenPup-M scores were associated with higher clinical examination scores indicating that there was an agreement between veterinary and owner assessment of mobility.

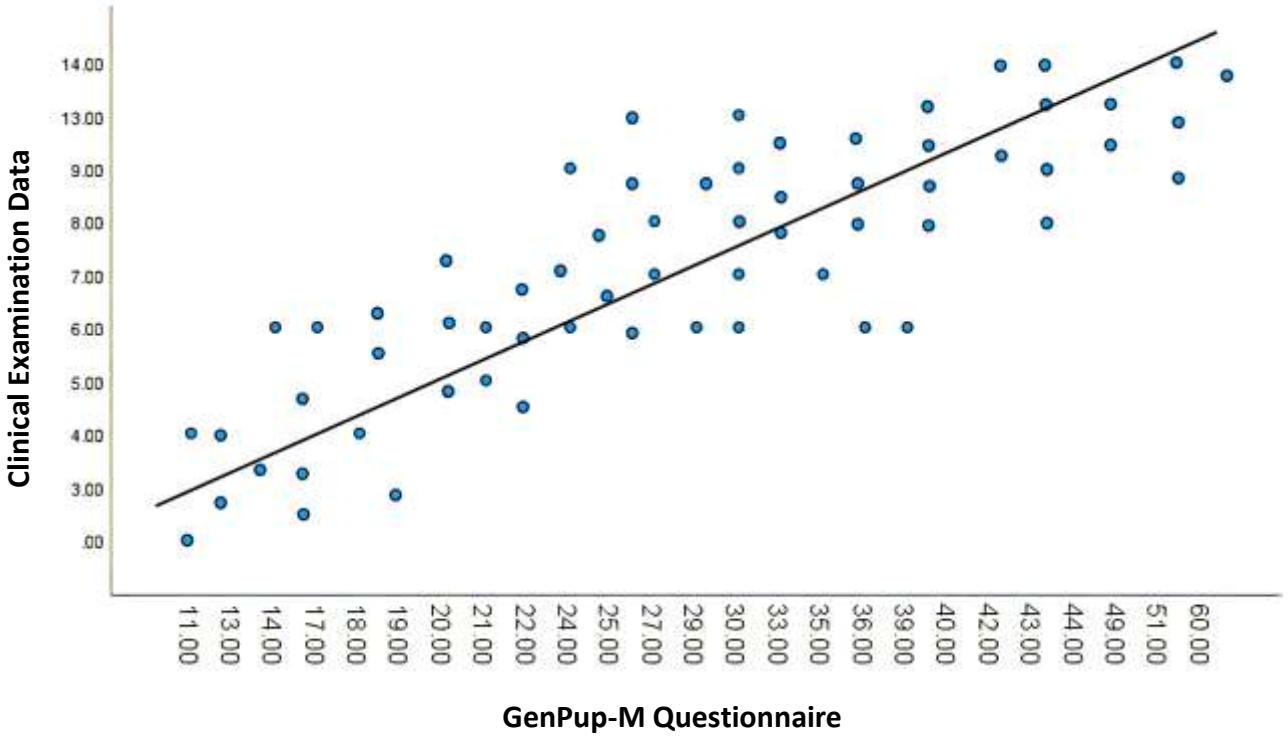

Figure 5. 2. Scatterplot of GenPup-M versus clinical examination data. There was a significant, moderate positive correlation typical of inter-instrument comparisons. Spearman's Rank Correlation Coefficient = 0.74 ($p<0.001$). A black line of best fit is shown through the scatterplot.

5.3.3 *Canine Gait Analysis*

As detailed in Section 4.2, a Spearman's rank correlation test was used to assess the relationship between GenPup-M responses and canine gait analysis parameters. Based on previous literature (Walton et al., 2013), PVF was determined to be the most useful to assess this correlation. The significance level was set at $p \leq 0.05$ (two-tailed). There was a moderate, positive correlation

between GenPup-M responses and PVF (r^2 = 0.43, p<0.001) (Taylor, 1990). Kendall's tau-b also showed a moderate positive correlation of 0.49 (p<0.001) between GenPup-M responses and PVF. The moderate positive correlation between GenPup-M and gait analysis is demonstrated on the scatter plot below (Figure 5.3).

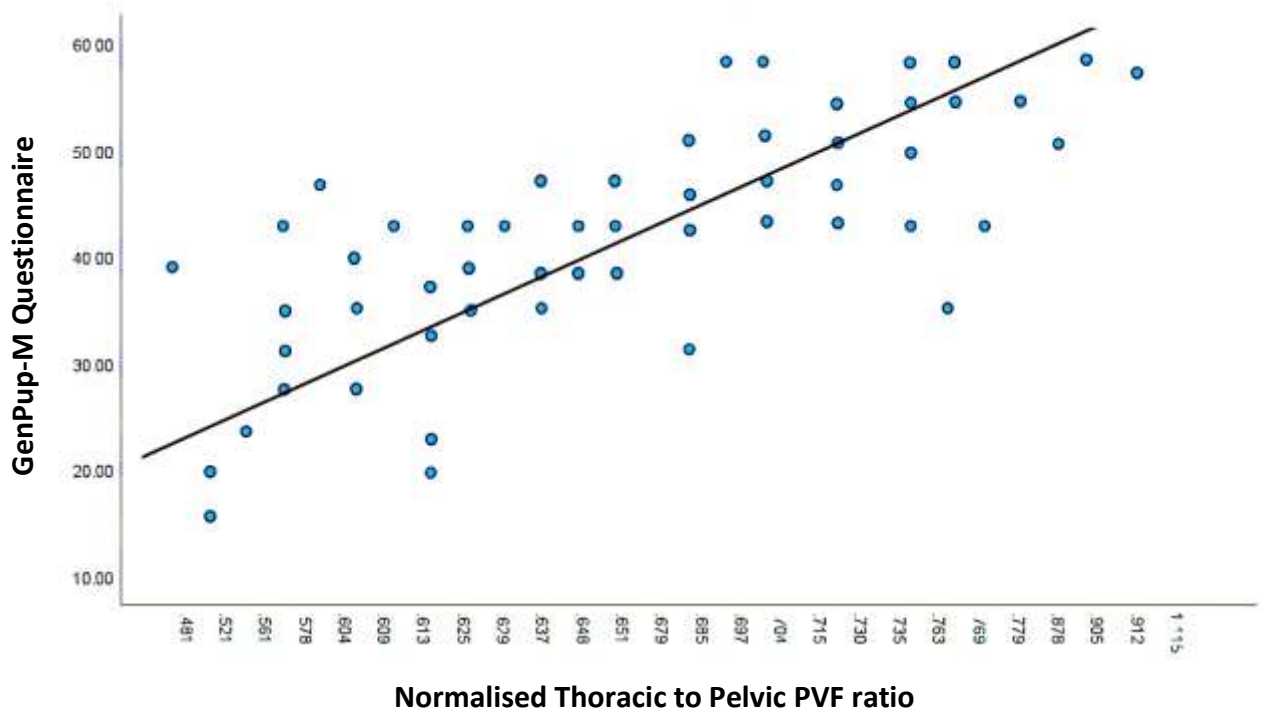

Figure 5. 3. Scatterplot of GenPup-M questionnaire scores versus thoracic and pelvic PVF ratio (using normalised values). There was a moderate positive correlation. Spearman's Rank Correlation Coefficient = 0.43 (p<0.001). A line of best fit is also shown on the scatter plot.

There was also a strong positive correlation between PVF and the overall validated veterinary clinical examination scores (r^2 = 0.51, p<0.001). Kendall's tau-b maintained the agreement between PVF and veterinary clinical examination moderate positive correlation of 0.50 (p<0.001) between veterinary examination scores and PVF.

5.4 Discussion

The LOAD questionnaire has previously been compared to other similar measures (CBPI and HCPI mobility questionnaires) to provide evidence of construct validity (Walton et al 2013). However, Walton et al were not the first to compare a CMI against similar validated questionnaires. One study tested construct validity by testing the newly designed instrument against a current validated CMI testing the same subject (Walton et al., 2013). Results from this study showed there were significant moderate correlations between LOAD, CBPI and HCPI CMIs, suggesting there is construct validity for all of the above instruments. In the present study, there was a significant relationship between total GenPup-M and LOAD scores. Spearman's correlation coefficient between GenPup-M and LOAD showed there was a significant moderate positive correlation for the inter-instrument comparisons. Although GenPup-M did not correlate as strongly as HCPI and CBPI with the LOAD questionnaire, as a previous study showed $r^2 = 0.74$ and $r^2 = 0.76$ respectively (Walton et al., 2013), this might be explained by questionnaire content, since GenPup-M is the first owner-reported mobility questionnaire aimed to detect all mobility impairments, including early on-set mobility impairments in dogs, whereas, LOAD, HCPI and CBPI are all designed to provide information relating to chronic canine mobility impairments or longitudinal analysis of specific orthopaedic conditions, e.g. elbow osteoarthritis. Thus, the objectives of GenPup-M and LOAD are somewhat different. However, according to Taylor, 1990, a strong correlation is any value above $r^2 = 0.68$, thus, all mobility questionnaires somewhat strongly correlate with one other and may prove useful when used in conjunction with each other to assess various aspects of canine mobility. Furthermore, the strong, positive correlation between the GenPup-M and LOAD questionnaire in this study supports good criterion validity and adds further evidence towards validation of GenPup-M.

Spearman's rank correlation coefficient was used to determine if the total clinical examination scores and GenPup-M responses were correlated, and thus, determine if owners' perceptions were in line with the veterinary judgement. There was a significant positive correlation between clinical examination scores and GenPup-M scores ($r^2 = 0.74$, $p<0.001$). Walton et al (2013) assessed criterion validity by comparing aggregate scores of the LOAD questionnaire and PVF by

using Spearman's rank correlation coefficients. Walton et al (2013) found that LOAD and PVF scores poorly correlated forces (r^2 = 0.23, p=0.01), however, still had statistical significance. GenPup-M had a strong positive correlation with PVF (r^2 = 0.43, p<0.001). These findings suggest that GenPup-M is more sensitive to detecting subtler mobility impairments, and correlates well with validated measures of canine mobility assessment. Furthermore, this project used a similar methodology to previous research (Muller et al., 2016; Walton et al., 2013; Hercock et al., 2009; Hielm-Björkman et al., 2003) whereby clinical history and clinical examination data were obtained to include/exclude participants in the study. The present study used the above information to categorise dogs into mobility impaired and non-mobility impaired cohorts, something which was perhaps controversial to previous literature, since it has been found that, with the exception of severe lameness, visual gait assessment was largely inaccurate (Waxman at al., 2008) and abnormalities can be detected in sound limbs (Evans, Horstman and Conzemius, 2005). However, the current study partially contradicts previous literature, since visual gait assessment only formed a small part of the clinical examination process and other factors were taken into consideration, for example, pain elicited when touching a joint, tissue swelling, muscle atrophy and crepitus, which may have been overlooked when using solely standalone quantitative gait assessment. Further limitations of using solely kinetic and kinematic gait assessment have been previously discussed in this thesis (Section 4.4.4).

5.5 Conclusion

GenPup-M positively correlated with all 'gold standard' measures of canine gait assessment, including a previously validated veterinary mobility questionnaire (LOAD), which previously, correlated well with HCPI and CBPI. However, as described above, the correlation between LOAD and GenPup-M was not as strong as with previous mobility questionnaires, perhaps due to the aim of the questionnaires being somewhat different with GenPup-M aiming to detect all mobility impairments, not just chronic conditions. As mentioned above, there was a moderately strong

positive correlation between GenPup-M and PVF, once again highlighting that kinetic gait analysis is more sensitive to mobility impairments than veterinary clinical examinations.

CHAPTER SIX | GENERAL DISCUSSION

6.1 Conclusion

There are many mobility impairments which can affect multiple joints including the elbow, carpal, tarsal, stifle and coxofemoral (hip) joints, but also, less commonly vertebral facet joints and metacarpophalangeal and metatarsophalangeal joints (Franklin, Park, Egger., 2009). Canine mobility impairments can negatively impact health and welfare, along with decreasing the dogs' lifespan (Anderson et al., 2018) and can sometimes prove challenging to detect and monitor longitudinally, especially in its early stages (Rychel, 2010). Thus, the implementation of non-invasive clinical scoring systems for use by veterinary surgeons and dog owners can assist in early preventative actions and minimally-invasive therapeutic interventions, such as hydrotherapy, acupuncture and/or physiotherapy (Kim, 2018). GenPup-M is the first owner-reported mobility questionnaire designed for all signs of canine mobility impairments and allows owners to assess their dog's behaviour in a routine environment during extended periods.

This study aimed to validate GenPup-M by using a veterinary clinical examination sheet (Harris et al., 2018), the LOAD questionnaire and quantitative canine gait analysis. 62 dogs (31 mobility impaired and 31 non-mobility impaired) were recruited in this study. A total of fifty owners completed the GenPup-M and LOAD questionnaire for a total of sixty-two dogs.

GenPup-M questionnaire responses were compared against the validated Liverpool Osteoarthritis in Dogs (LOAD) questionnaire (Walton et al., 2013), kinetic and kinematic gait analysis, and a validated veterinary clinical examination (Harris et al., 2018) using Spearman's rank correlations to test criterion and construct validity. Principal Component Analysis (PCA) was used to identify if one or more question components could predict the absence/presence of mobility impairments. Cronbach's α was used to test internal consistency of GenPup-M. The PCA identified two components with Eigenvalues >1 ("stiffness/ease of movement" and "willingness to be active/exercise"). Cronbach's α was "good" (0.87). There was a strong, positive correlation between GenPup-M and LOAD responses ($r^2 = 0.69$, $p<0.001$). GenPup-M correlated with the LOAD questionnaire similarly to previously validated mobility questionnaires; Walton et al., 2013

found that the HCPI and CBPI positively correlated with the LOAD questionnaire (a Spearman's rank correlation showed $r^2 = 0.74$ and $r^2 = 0.76$ respectively). Although the correlation between GenPup-M and LOAD was still considered strong (>0.68 = strong correlation (Taylor, 1990)), it was not as strong as the HCPI and CBPI, perhaps this was due to the LOAD, HCPI and CBPI being designed to provide information relating to chronic canine mobility impairments and longitudinal monitoring, conversely, GenPup-M is designed to detect all mobility impairments, including early on-set changes in mobility.

No previous veterinary studies have investigated the correlation between CMIs and clinical examination. The present study found that GenPup-M and clinical examination scores returned the strongest, positive correlation ($r^2 = 0.74$, $p<0.001$) compared to LOAD and PVF. A validated clinical examination sheet (Harris et al., 2018) was considered the gold-standard method of determining mobility impairments for this cohort since all dogs were categorised as either mobility impaired or non-mobility impaired based on clinical examination results. The strong correlation was considered a positive finding as it is our aim that GenPup-M will be mostly used alongside clinical history and clinical examination in small animal practice.

There was a moderate, positive correlation between GenPup-M responses and Peak Vertical Force (PVF). ($r^2 = 0.43$, $p<0.001$). Walton et al (2013) also assessed construct validity by comparing aggregate scores of the LOAD questionnaire and PVF by using Spearman's rank correlation coefficients, the LOAD and PVF were found to have a poor correlation ($r^2 = 0.23$, $p=0.01$) to once another. Perhaps, due to the GenPup-M questionnaire being more sensitive to detecting more subtle changes in canine gait, which occasionally corresponded with gait analysis. However, above Spearman's rank results for GenPup-M positively correlate with all the objective forms of gait assessment (quantitative 3D gait analysis, validated veterinary clinical examination and a validated canine mobility questionnaire (LOAD)), adding further evidence towards validation of GenPup-M for use in all canine mobility impairments.

Quantitative canine gait analysis showed there were statistically significant differences between peak vertical forces (PVF) of mobility impaired and non-mobility impaired dogs ($p<0.05$). Olson and Carithers, 1982 created the concept that dogs unevenly distribute their weight onto thoracic

and pelvic limbs (60:40%, respectively); analyses of PVF showed that dogs with mobility impairments more evenly distributed their weight across all four limbs, compared to non-mobility impaired dogs. There were also variations in spatiotemporal parameters; stance time and swing phase were generally lower for the mobility impaired cohort, compared to the non-mobility impaired cohort, suggesting that mobility impaired dogs were walking slower than non-mobility impaired dogs.

Therefore, in summary, this study found that GenPup-M has positively correlated with gold standard objective forms of gait assessment (validated clinical examination, quantitative gait analysis and previously validated mobility questionnaire), adding evidence towards its validation. It is hoped GenPup-M will be distributed into commercial veterinary practices for completion by owners at yearly vaccinations/health checks, allowing veterinary professionals to collect a large amount of data and (subject to owner consent) allow further investigation into early-onset canine mobility impairments. Furthermore, the University of Liverpool and Dogs Trust are also working in conjunction to try and develop GenPup-M into a smartphone App to facilitate rapid owner-/primary carer-reported mobility assessment. There is also scope to further the use of GenPup-M to collect information relating to risk factors (e.g. walk duration/frequency, body condition score (BCS)) associated with early-onset canine mobility impairments (see further details in the Future Work section (Section 6.3)).

6.2 Limitations

6.2.1 *Breed*

Here, there was large heterogeneity among the sample with respect to size, body weight and breed, whereas other gait studies have focused on more homogenous groups such as Labrador retrievers (Gillette and Zebas, 1999; Evans et al., 2005; Evans, Horstman and Conzemius, 2005 and Smith et al., 2006; Hercock et al., 2009) or Greyhounds (Besancon et al., 2004; Roush and McLaughlin, 1994; Bertram et al., 2000). There was a consensus among these studies that variance was attributable to individuals when comparing different breeds and identical breeds

(Jevens et al., 1993; Kim et al., 2011; Voss et al., 2011). Thus, morphology and conformation can be highly influential in interpreting results from canine gait analysis. This may have impacted the current study as there were 17 different breeds represented in the sample, ranging from Jack Russell Terriers to a Great Dane. This heterogeneity likely caused large magnitudes of variation within the gait dataset.

Very few studies investigating mobility impairments have included dogs under 10kg (Kim et al., 2011; Evans, Horstman and Conzemius, 2005). Thus, these breeds are underrepresented when compared to medium and larger breeds. Smaller breed dogs proved challenging to analyse in this study; 16 dogs <10kg were recruited in this study, six dogs had to be discarded due to being too small to gain accurate PVF readings from the force plates. Additionally, for the discarded six dogs, the spatiotemporal data was inaccurate due to the markers being very close together, thus, when data was being cleaned in Qualisys Track Manager (QTM), markers were hard to correctly identify and kept swapping anatomical names. Fur interference with the retro-reflective markers was a large problem throughout the study, particularly on the foot, however, in larger breeds, these markers were further spread apart and easier to identify. Furthermore, specific force plate settings had to be maintained for smaller dogs (<5kg) undergoing gait analysis to yield accurate PVF. The discarding of data from very small dog breeds within the project means that there is a reduced ability to generalise the results of your work to smaller breeds of dogs, further contributing to the problem of underrepresentation of small dogs within gait analysis studies.

6.2.2 *Impact of COVID-19*

Recruitment of dogs during COVID-19 was reduced to a very small demographic of dog owners. As most dog owners were recruited from within the University of Liverpool, the study sample size contained a population of dog owners from a limited cross-section of socio-economic backgrounds. Many of the dog owners from within the institute worked in musculoskeletal and ageing science, and thus, will have had a greater understanding/more experience of mobility impairments compared to the general public. Hence, the limitation is based on whether these

owners are likely to be representative of the general dog-owning public and how the GenPup-M will have been completed/ability to report subtle changes in exercise levels and mobility. To try and mitigate this bias and widen the diversity, additional dogs were recruited from within social circles of NC, alongside cleaners and technicians from within the WHD building to apply the results of this project to the wider population of dog owners. Future projects, COVID-19 allowing, will recruit dog owners as originally intended from Small Animal Practice and Leahurst Teaching Hospital.

Many dog owners from outside the institute were not allowed to enter the WHD Building during lockdowns, which created some hesitancy to participate due to unwillingness to leave their dog(s) alone. Furthermore, some dogs experienced separation anxiety, subsequently, data collection had to be aborted due to the dogs refusing to trot/walk over the force plates without their owners present.

6.2.3 *The Use of Other Systems to Detect Mobility Impairments*

Three-dimensional kinematic systems often require multiple motion capture cameras to achieve movement detection in a large space. Consequently, this is often restricted to specialised gait laboratories and universities. Additionally, motion capture camera systems and force plates are expensive and require training to operate. Thus, access to these methods of gait analysis is not freely available for small animal practices. Alternatively, pressure platforms are often used in veterinary settings (Oosterlinck et al., 2011; Besancon et al., 2003; Schnabl-Feichter et al., 2020) as they require less space compared to floor-mounted force plates and do not involve specialist camera equipment. Pressure platforms are relatively user-friendly and portable, they can provide multiple readings for useful parameters to aid the diagnosis of complex mobility conditions from a single pass and there are no size restrictions. However, these platforms do not allow analysis of kinematic data, thus, would not have been useful in this project.

As mentioned in Section 1.1.3, radiography is deemed the mainstay of diagnostic imaging for clinicians to investigate orthopaedic concerns in small animal practice (Fujita et al., 2005) as it is fairly sensitive to anatomical changes which occur as a result of mobility impairments. However, radiography was not undertaken during the study due to animals requiring a general anaesthetic to obtain diagnostic images. Each general anaesthetic carries an associated risk leading up to, and including death. Furthermore, a validated clinical examination sheet, combined with kinetic and kinematic gait analysis was deemed sensitive enough to determine if a dog had a mobility impairment, hence, the increased risk of a general anaesthetic to undertake radiography was not considered necessary for this project to achieve the objectives. Similarly, not all mobility impairments may be suitable for radiographic imaging as orthopaedic changes may not always equate with clinical evident orthopaedic pain (Widmer et al., 1994; Dendrick et al., 1993), for example, cartilage degradation is not visible, nor are some mild changes of orthopaedic disease (Allan and Davies, 2018). Furthermore, since this project aimed to validate a questionnaire that claims to detect all mobility impairments, more sensitive imaging modalities may have been required to identify clinically significant lesions. Magnetic Resonance Imaging (MRI) may have provided evidence for structural or compositional cartilage changes, when perhaps, the orthopaedic disease is asymptomatic. Computerised Tomography (CT) may have helped to identify later stages of the disease when microscopic bone remodelling or osteophytosis occurs (Jones, Pitsillides and Meeson, 2022). However, given the scope of the project, MRI or CT imaging was not considered reasonable or feasible. Moreover, if mobility impairments are due to breed conformation, e.g. Brachycephalic Obstructive Airway Syndrome (BOAS), imaging will be of no use, since orthopaedic structures may look anatomically normal for that specific breed.

6.3 Future Work

There will be several uses for the validated GenPup-M questionnaire, however, one of these is to distribute GenPup-M into commercial veterinary practices for completion by owners at yearly vaccinations/health checks. If dogs' owners completed the GenPup-M annually, veterinary

researchers would have access to a large amount of data (subject to owner consent), to enable future investigation into canine mobility impairments. Thus, improving canine health and welfare.

GenPup-M, like many other mobility CMIs, can assess natural canine behaviour in a home environment during extended periods of time. GenPup-M is easy for the owner to use, patient-centred and requires no specialised equipment. Belshaw et al (2018) stated that coaching owners to evaluate behavioural indicators of pain at home takes a great deal of time, a commodity which is in short supply in general practice. The use of tailored CMIs reduces this time while offering the possibility of longitudinal monitoring of a dog's condition. Additionally, canine behavioural changes which are associated with pain are often subtle and occur gradually as chronic pain persists. Hence, many owners are completely unaware that there is a problem until more advanced signs develop.

Both NC and EC have been in collaboration with the UoL and Dogs Trust to develop GenPup-M into a smartphone application for owners to use. There are many veterinary based Apps on the market that allow owners to continuously monitor their pets' health conditions, these include Royal Veterinary College Pet Diabetes, DogLog and FitBark. However, currently, there is no App centred on mobility monitoring. Developing GenPup-M into an App would provide owners with an easily accessible format to complete the questionnaire. Furthermore, by using the App, owners would have the opportunity to compare their pet's most recent results with those of previous months/years (enabling longitudinal monitoring) of their dog's health. Consequently, owners could share data with their veterinary surgeon to discuss any subtle mobility changes, enabling early interventions to be made. The development of the App would make a positive contribution to the dog-owning population, veterinary profession and improve canine health and welfare. It is estimated up to 80% of dogs suffer from mobility impairments by the age of 8 years old (Anderson et al., 2018), and most dogs only present to veterinary practices when they are visibly lame to their owners. As mentioned above, Belshaw et al (2018) stated teaching owners to detect subtle mobility changes is laborious. However, if owners routinely completed a validated questionnaire (with its primary focus to detect early onset mobility impairments), there is a higher chance that these changes will not go unnoticed.

Preliminary data from this project had prompted the research team (NC, EC, KB, JM and LH) to successfully secured funding for a PhD grant (funded by a Dogs Trust Canine Welfare Grant) to further the work of this MPhil. The approved project aims to extend data collection from this existing group of dogs (n=62) to examine longitudinal changes in mobility across a three-year period. During the proposed project, the existing dataset is planned to be extended by collecting the mobility data at yearly timepoints for an additional 60 young dogs from a breed (Labrador retriever) at an increased risk of mobility problems. Both dog groups (n=127) will form the University of Liverpool (UoL)-GenPup-M mobility cohort. Since this PhD application is in collaboration with the Dogs Trust GenPup team, the existing GenPup-M responses and detailed owner-reported longitudinal data will be used to study information (subject to owner consent and a data-sharing agreement in place) relating to risk factors (e.g. walk duration/frequency, body condition score (BCS)) associated with early-onset canine mobility impairments.

A review article on the use of scoring systems in the detection of canine mobility impairments has been submitted by NC to the Journal of Small Animal Practice (JSAP) for peer review. An abstract detailing the results of this project has been approved for oral presentation at the British Small Animal Veterinary Association (BSAVA) Congress in 2022. Once this MPhil thesis has been submitted, data will be compiled into individual publications detailing the results of the GenPup-M validation, and quantitative canine gait analysis, including joint kinematics, step-to-step variation in kinematics to determine if mobility impaired dogs are more or less variation. There is also scope to assess mobility variation using different terrains (e.g. sand, concrete, grass) to see how this impacts spatiotemporal parameters.

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SUPPLEMENTARY MATERIAL

S1: Owner information sheet

S2: Owner consent form

S3: COVID-19 consent form

S4: Veterinary clinical examination sheet

S5: Veterinary clinical examination sheet key

S6: An additional clinical examination sheet was provided by EC to ensure all aspects of the orthopaedic and neurological assessment were completed in a methodical order. This checklist was used in conjunction with supplementary material 5 but acted as a teaching aid for NC.

S7: GenPup-M questionnaire

S8: Retro-reflective marker positions

S9: Data for all mobility impaired and non-mobility impaired dogs, containing values for Peak Vertical Force (PVF) (normalised), and non-normalised spatiotemporal values, including cycle time, duty factor, stance time, swing time, stride length, step width. Non-normalised average speed is also included

S1

INSTITUTES OF LIFE COURSE AND MEDICAL SCIENCES AND SCHOOL OF SCIENCE, UNIVERSITY OF LIVERPOOL

Validation of a questionnaire used to determine mobility in an ongoing longitudinal canine ageing study (Dogs Trust Generation Pup)

Invitation and summary:

We would like to invite your dog to participate in a research study funded by the Dogs Trust. Before you decide whether or not to participate, it is important for you to understand why the research is being done and what it will involve: this is the purpose of this document. Please take time to read the following information carefully and feel free to ask us if you would like more information or if there is anything that you do not understand. Please also feel free to discuss this with your friends, relatives and GP/vet if you wish. We would like to stress that you do not have to accept this invitation and should only agree to take part if you want to.

The first part of the Participant Information Sheet tells you the purpose of the study and what will happen to your dog if you take part. Then we give you more detailed information about the conduct of the study where we explain the potential risks and benefits involved in participation in the study.

Thank you for reading this.

What is the purpose of this study?

This project will test the agreement between a new owner-based questionnaire on movement of dogs used in the lifetime study "Dogs Trust Generation Pup", and veterinary orthopaedic examination and accurately measured (objective) motion/gait data. Agreement of these measurements and therefore validation of this questionnaire will identify risk factors for reduced movement/mobility thereby contributing to early management of diseases affecting mobility and improved canine welfare in veterinary practice.

Owner-based questionnaires verified with objective gait data, where we walk or trot dogs across a metal plate in the floor which gives us accurate scientific measurements on the load the dogs

feet place as they move, are available for use in dogs already diagnosed with osteoarthritis to help accurately quantify changes in mobility. However, declines in canine mobility can be gradual and dogs are not always presented to veterinary surgeons for early assessment. Currently, there is no validated owner-based questionnaire to determine these subtler mobility changes.

Our group have worked with the Generation Pup team (https://generationpup.ac.uk) developing a questionnaire ("GenPup-M") to collect mobility data from dog owners at repeated time points. However, accurate scientific validation of "GenPup-M" has not been performed for use in either the Generation Pup or general dog populations. This is essential prior to detailed analysis and publication of risk factors for owner-reported canine mobility problems.

We will assess the accuracy of owner-responses to "GenPup-M" questions in dogs with/without mobility problems using independent veterinary assessments and gait analysis.

Why have I been chosen to take part?

We aim to recruit dogs either owned by University of Liverpool Staff members or presented for routine appointments at the University of Liverpool (UoL)'s Small Animal Practice and at the UoL Small Animal Teaching Hospital. We will include all dogs over 5 months old if we (veterinary surgeons) and you (the owner) have no concerns about the dog being subject to an independent veterinary assessment for mobility and/or gait analysis, on the basis of health and/or behaviour concerns.

Do I have to take part?

Your participation is voluntary and you are free to withdraw at any time without explanation and without incurring a disadvantage. If you wish to withdraw please feel free to let us know by contacting the principal investigator [Professor Eithne Comerford: ejc@liverpool.ac.uk].

What will happen if I take part?

If you are happy to participate in the study,

1) You will be firstly asked to fill in a questionnaire online about your dog's mobility (Gen-Pup-M)(Appendix 1) prior to attending the practical part of this study.
2) Your dog will have a general clinical, orthopaedic and neurological examination carried out by the veterinary qualified Masters student, Dr. Clark, who is funded by the Dogs Trust on this project, under the supervision of the principal investigator, Professor Eithne Comerford.
3) Your dog will have its gait examined on a force plate or pressure platform at either the Small Animal Teaching Hospital or the William Henry Duncan Building at the University of Liverpool. Dogs will be asked to walk with around 20 small infrared markers and sensors attached to the skin with sticky tape. A special camera system will track the motion of

infrared markers attached to the skin (a small section of your dog's fur may be clipped to allow the markers to be visible to the cameras). These cameras only record the motion of the markers and do not record standard video. Dogs will walk for a short distance (approximately 5 metres) along a trackway that includes a force plate/ and or pressure platform which is embedded beneath the floor. This process will be repeated until 10 successful continuous bouts of walking/trotting have been recorded.

The questionnaire and veterinary examinations will be performed by the Masters student or PI. The gait analysis data will be collected by the research team which may include the Masters student, PI and trained technicians/nurses from the Institutes of Ageing & Chronic Disease's and Veterinary Sciences gait laboratories.

Your dog's welfare is paramount in this study, therefore, throughout his/her time spend participating in the study he/she will have access to fresh water and rest breaks, please feel free to bring additional treats for your dog to enjoy.

The investigators are happy to elaborate on any details and answer any questions you have regarding the experiment.

What are the risks in taking part?

As the examinations are part of routine veterinary practice and the gait analysis data collection is non-invasive, there is very little risk to you or your dog. In the walking/trotting performed as part of the gait analysis data collection, there is a theoretical risk that your dog may trip or fall, but these risks are no greater than during everyday life.

Will I benefit directly from taking part?

We cannot offer any payments, as this study does not have any direct funding to cover day-to-day experimental costs. There are no direct or immediate benefits to you or your dog from taking part other than participation in a study that may inform you about how your dog walks. In the medium to long term we hope this research will better inform veterinary surgeons about normal dog mobility with the view to spotting any problems earlier which may allow quicker treatment for any musculoskeletal issues. All participants taking part in this and other University of Liverpool studies are covered for negligent and non-negligent harm by the University's Clinical Trials Insurance policy. We are unable to offer any veterinary advice during this study and advise to seek your own vet if you have any medical concerns regarding your pet.

What will happen to the results of the study?

When are sufficient data to draw conclusions, we will submit the results for peer-reviewed publication in open access veterinary scientific literature so that owners and veterinary surgeons

around the world have access to the conclusions. No personal information collected from you or your dog will appear in any reports or papers. At the end of the study, you will be invited to a virtual debriefing session where we will present our results, if you wish to attend.

How will my data be used?

The University processes personal data as part of its research and teaching activities in accordance with the lawful basis of 'public task', and in accordance with the University's purpose of "advancing education, learning and research for the public benefit.

Under UK data protection legislation, the University acts as the Data Controller for personal data collected as part of the University's research. The Principal Investigator, Professor Eithne Comerford, acts as the Data Processor for this study, and any queries relating to the handling of your personal data can be sent to Professor Comerford's details as listed below

Further information on how your data will be used can be found in the table below:

How will my data be collected?	Your personal data will be inputted onto a password protected University SharePoint site to which only the PI and Masters student will have access.
How will my data be stored?	All personal data will be stored on the SharePoint site and any analysed data will be anonymised and will be kept on a password protected University drive which is only accessible by the PI/Masters student.
How long will my data be stored for?	5 years
What measures are in place to protect the security and confidentiality of my data?	All data will be kept on a password protected University drive which is only accessible by the PI Masters student. No data will ever be held on a mobile laptop or non-University networked computer.
Will my data be anonymised?	Yes for all data processing and analysis
How will my data be used?	The anonymised data will be used to validate the Gen-Pup-M questionnaire with the force plate data to develop an open access mobility tool for vets.
Who will have access to my data?	Only the PI and Masters student.

Will my data be archived for use in other research projects in the future?	No – it will only be used in this study.
How will my data be destroyed?	It will be deleted from the SharePoint site after 5 years.

The University strives to maintain the highest standards of rigour in the processing of your data. However, if you have any concerns about the way in which the University processes your personal data, it is important that you are aware of your right to lodge a complaint with the Information Commissioner's Office by calling 0303 123 1113.

What will happen if I want to stop taking part?

You should feel under no pressure to participate and you will be free to withdraw at any time. You do not need to explain withdrawing. Results up to the period of withdrawal may be used, if you are happy for this to be done. Otherwise you may request that they are destroyed and no further use is made of them. If you wish to withdraw please feel free to let us know by contacting the principal investigator [Professor Eithne Comerford: ejc@liverpool.ac.uk].

How do I participate in the study?

If having considered all the information provided, and obtained satisfactory answers to any questions about participation, you would like to participate in the study then you will be asked to sign a consent form. Signing this consent form serves as written confirmation that you understand the requirements, risks and benefits of participation and that to the best of your knowledge you do not violate any of the exclusion criteria stated herein for this study.

What if you are uncertain or have further questions?

If you are unhappy, or if there is a problem, please feel free to let us know by contacting [Professor Eithne Comerford: ejc@liverpool.ac.uk / 07807106568] and we will try to help. If you remain unhappy or have a complaint which you feel you cannot come to us with then you should contact the Research Ethics and Integrity Office at ethics@liv.ac.uk. When contacting the Research Ethics and Integrity Office, please provide details of the name or description of the study (so that it can be identified), the researcher(s) involved, and the details of the complaint you wish to make.

Investigator:

Professor Eithne Comerford, Principal Investigator

For queries or more information about any of the information below contact the principal investigator via email (ejc@liverpool.ac.uk) or by telephone (07807106568).

S2

UNIVERSITY OF LIVERPOOL

CONSENT FORM

Title of Research Project:	**Validation of a questionnaire used to determine mobility in an ongoing longitudinal canine ageing study (Dogs Trust Generation Pup)**
Researcher(s)	Professor Eithne Comerford and Dr. Natasha Clark

Please initial box

1. I confirm that I have read and have understood the information sheets dated 090920 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected. ☐
3. I understand that the data will be stored in accordance with the UK data protection act and under this act, I can at any time ask for access to the information I provide and I can also request the destruction of that information if I wish. ☐
4. I agree to take part in the above study. ☐

Participant Name (print)	Participant e.mail (required)	
Date (dd/mm/yyyy)	Signature	
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

S3

INSTITUTE OF LIFE COURSE & MEDICAL SCIENCES and SCHOOL of VETERINARY SCIENCE, UNIVERSITY OF LIVERPOOL

Dogs Trust Generation Pup: Validation of owner-reported mobility data using a tool developed for the Generation Pup longitudinal study (GenPup-M)

Participant Information Sheet on COVID-19 Risk (14/08/2020)

Investigators:

Professor Eithne Comerford, Principal Investigator

Dr Karl Bates, Co-Investigator

Mr Andrew Tomlinson, Co-Investigator

Dr Natasha Clark, Co-Investigator and MPhil Student

For queries or more information about any of the information below contact the principal investigator via email (ejc@liverpool.ac.uk) or telephone (mobile: 07807106568; office: 0151 7942000).

Participating in this study will mean encountering circumstances that slightly increase the risk of contracting COVID-19. The greatest increase in risk is likely to come from your commute to the University of Liverpool facilities, and this risk will vary according to your route and mode of travel. Please see the government guidance on travel to work (https://www.gov.uk/guidance/coronavirus-covid-19-safer-travel-guidance-for-passengers).

While participating in this study you will visit two facilities within the University of Liverpool. Both these facilities are operating University approved COVID-safe procedures, including social distancing, bubble systems for staff, and provision of PPE. However, any close contact with other people inherently increases the risk of COVID-19 transmission, and therefore there is a small increased risk associated with your participation. All university staff are under strict instructions to self-isolate and seek a COVID-19 test should they begin to experience symptoms. If one of the investigators experiences symptoms before you are due to participate then they will cancel your

visit to the university. We also ask that you provide us with your contact details (name, telephone number and email address) so that we can contact you should an individual that you have come into contact with during your participation contact COVID-19 around the time of your visit to the university.

PLEASE TICK BOX

1. I confirm that the paragraph above outlining the increased risk of contracting COVID-19 as a result of participation in this study was provided to via email and/or telephone prior to my visit to the University of Liverpool, and I am receipt of this printed version again prior to the commencement of participation. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I confirm that consent to my contact details (name, telephone number and email address) be retained by the investigators so that they can contact me if necessary in the advent of a relevant contraction of COVID-19. ☐

Participant Name	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

Participant contact details

NAME:

TELEPHONE NUMBER:

EMAIL ADDRESS:

S4

Case no.: .....

Clinical Checklist for Canine Osteoarthritis

Clinician: .....

Owner Name: ..... Dog Name: .....

1. General

Age, breed, sex, neuter status:
.....

Weight and Body Condition Score (see attached key): .....

OA history (brief summary; time of onset, signs of disease noticed by owner, analgesic drugs currently prescribed, when drugs were last taken etc.):
.....
.....
.....
.....
.....

Other health related issues (inc. **eye test**- blink reflex, tracking and obstacles)
.....
.....
.....

2. Mobility

A) Lameness during locomotion (0-10 NRS, See guidelines in Vasseur and Slatter 1993)

- Overall Lameness score: .....
- Limbs affected: .....

B) Ability to:

- Stand up: Score (0-3): .....
- Lie down: Score (0-3): .....

3. Physical examination

Fore limbs

	Left		Right	
	☺	☹	☺	☹
Digital joints				
Carpus				
Elbow				
Shoulder				

Hind limbs

	Left		Right	
	☺	☹	☺	☹
Digital joints				
Tarsus				
Stifle				
Hip				

JFS and Abnormalities (see key for scores)

Joint Affected →										
Abnormalities (0/1)↓	L	R	L	R	L	R	L	R	L	R
Crepitus										
Tissue swelling										
Effusion										
Abnormal bone/ joint structure*										
Muscle atrophy**										
Inc. temperature										
JFS (0-4)										

---

5. Global Score

Overall judgement of severity of the animal's condition (tick correct)

	Owner	Clinician	Student
None			
Mild			
Moderate			
Severe			

S5

KEY

Body Condition Score (BCS): (LaFlamme 1997)

1. Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss of muscle mass.

2. Ribs, lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence. Minimal loss of muscle mass.

3. Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist.

4. Ribs easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident.

5. Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed.

6. Ribs palpable with slight excess fat covering. Waist is discernible viewed from above but is not prominent. Abdominal tuck apparent.

7. Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and base of tail. Waist absent or barely visible. Abdominal tuck may be present.

8. Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck. Obvious abdominal distension may be present.

9. Massive fat deposits over thorax, spine and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distention.

Lameness score (adapted from Vasseur and Slatter (1993) In. Text Book of Small Animal Surgery, 2nd Edition)

0: Sound

1: Occasionally shifts weight

2: Mild Lameness at slow trot, none while walking

3: Mild lameness while walking

4: Obvious lameness when walking, but places foot when standing

5-8: Increasing degrees of severity between 4 and 9

9: Places toe when standing, carries limb when trotting

Lameness score (stand up/ lie down): 0= normal function, 1= quite slow/stiff, 2= very slow/stiff, 3= unable to perform action without assistance

Joint abnormality score

0= absent, 1= present

Joint function score (JFS) (adapted from Impellizeri 2000)

0- Normal range of motion, no stiffness evident and no pain response during manipulation
1- One of the following joint defects:
Reduced range of motion
Pain response to flexion
Pain response to extension
Pain response to other manipulation e.g. abduction (hip and shoulder), internal rotation (elbow), "drawer test" (stifle) etc.
2- Two of the joint defects described in score 1
3- Three/four of the joint defects described in score 1
4- Three/four of the joint defects described in score 1 **and** pain response to touching joint or limb/guarding limb.

>Pain response here may refer to vocalisation (e.g. yelp or whine), avoidance behaviour (e.g. struggling) aggressive behaviour, etc.

* e.g. Patella luxation, roughness of cortices etc

** (of associated limb)

S6

Orthopaedic Clinical Examination Checklist

- Visual gait analysis, if there is any ataxia, turn in tight circles.
- Check the head
    - Neurological examination to check facial nerves and intact menace
    - Move the neck and head up/down, side to side looking for signs of pain
    - Check in mouth and teeth
    - Check lymph nodes
- Palpate the cervical vertebrae
- Feel down both thoracic limbs for symmetry and effusion (particularly around the elbow)
    - Start to look at paw pads
    - Flex and extend the digits
    - Flex and extend the carpus
    - Palpate the long bones of the thoracic limb
    - Apply force to extend the elbow by using your hand on the back of the elbow
    - Internally and externally rotate the forearm
    - Internally and externally rotate the shoulder
    - Flex and extend the shoulder
    - Test knuckling reflexes bilaterally
- Palpate along the spine
- Palpate the abdomen
- Palpate the hips and feel for the landmarks, check for asymmetry
- Feel for asymmetry and palpate the stifle, feel for effusion
- Palpate the patella ligaments – both should feel like pencils
- Feel down the hock, across the calcaneus and malleolus, down to the toes
    - Start to look at paw pads
    - Flex and extend the digits
    - Flex and extend the tarsus
    - Palpate the long bone
    - +/- tibial thrust at the stifle: extend the stifle and flex the hock
    - Flex and extend the stifle
    - Extend and flex the hip
    - Test knuckling reflexes bilaterally
- Flex the hips and put your chin in the lumbo-sacrum
- +/- Tail jack, +/- assess anal tone

S7

‘Five month’ questionnaire

This questionnaire will allow us to find out about your dog's recent experiences with you. It is designed to be completed when your dog is approximately 5 months old.

Thank you in advance for your help with this survey.

Date when this questionnaire was completed

Insert date below…

_____

Please write details below if you have had any changes in your contact details since the last questionnaire

Step 1 of 2...How is your puppy?

1 How is your puppy in him/herself?	
	Tick one box
Very well	☐
Fairly well	☐
Satisfactory	☐
Poorly	☐
Very poorly	☐

Step 2 of 2...Mobility and Exercise

Section 1 – Mobility

1 Does your puppy currently have ANY mobility problems, for example occasional stiffness when getting out of bed, difficulty jumping into the car/onto a bed or sofa?

I'm unsure	☐
Yes	☐
No	☐

1.a ****If you ticked 'I'm unsure' or 'No' in Q.1 please answer this question and then move Q.2****

Is your puppy currently receiving any medication/dietary supplements/other therapies or treatments to help him/her with his/her mobility?

	Tick one box
I'm unsure	☐
Yes	☐
No	☐

1.b ****If you ticked 'Yes' in Q.1 please answer this question, otherwise move to Q.3.a****

How old was "Toby when he started having his/her mobility problems?

	Tick one box
Not applicable - never had mobility problems	☐
1 month or less	☐
2 months	☐
3 months	☐
4 months	☐
5 months	☐
I don't know/can't remember	☐
Other (please specify) _____	☐

2 If you can, please list below any medications that your puppy is currently receiving for mobility problems.

3 If you can, please list below any medications that your puppy is currently receiving for problems other than mobility.

4 Please list any dietary supplements that your puppy is currentlyreceiving.

5 Since I have owned him/her, my puppy has had…

Tick one box per row

	Never	Yes, in the past, but not currently	Yes, currently receiving	I don't know/can't remember
Acupuncture	☐	☐	☐	☐
Hydrotherapy	☐	☐	☐	☐
Physiotherapy	☐	☐	☐	☐
A massage/massages	☐	☐	☐	☐

6 Please use this space to tell us about any other treatments (for example stem cell therapy, laser, shock wave treatment) that you have used/are using to help your puppy with mobility problems…

_____

_____

_____

_____

_____

_____

_____

Section 2 – Lifestyle

7 In the last week, <u>on average</u>, how far has your puppy exercised each day?

	Tick one box
Less than a mile	☐
Between 1 mile and just under 2 miles	☐
Between 2 miles and just under 3 miles	☐
Between 3 miles and just under 4 miles	☐
4 or more miles	☐
I don't know/can't remember	☐
Other (please specify) _____	☐

8 Are there particular days of the week upon which your puppy has significantly more exercise?

	Tick all that apply
Mon	☐
Tues	☐
Weds	☐
Thurs	☐
Fri	☐
Sat	☐
Sun	☐

9 My puppy is exercised most often over the following sort of terrain…

	Tick a maximum of two
On level grass	☐
In woodland	☐
On roads/pavements	☐
Over rough hill ground	☐
On sand	☐
On pebbles	☐
Other (please specify)_____	☐

10 At exercise, my puppy usually spends most of his/her time…

	Tick one box
Walking (on a long/flexi-lead)	☐
Walking (on a short lead)	☐
Walking (off lead, 'to heel')	☐
Walking (off lead, not 'to heel')	☐
Trotting (on a long/flexi-lead)	☐
Trotting (on a short lead)	☐
Trotting (off lead, 'to heel')	☐
Trotting (off lead, not 'to heel')	☐
Running freely (faster than a trot)	☐
Other (please specify) _____	☐

11	**Does your puppy have his/her activity levels restricted when he/she is exercised?**	
		Tick one box
	No - he/she can run around as much as he/she likes off lead	☐
	Only if he/she looks tired and/or stiff/lame	☐
	Yes - the walker limits his/her exercise	☐
	Other (please specify) _____	☐

Section 3 – General Mobility

12 How much do you think your puppy's mobility adversely influences his/her general well being?

Please circle the answer for the question above…

Not applicable	*Not at all*					*Moderately*					*Extremely*
n/a	0	1	2	3	4	5	6	7	8	9	10

13 How disabled is your puppy by lameness?

Please circle the answer for the question above…

Not applicable	*Not at all*					*Moderately*					*Extremely*
n/a	0	1	2	3	4	5	6	7	8	9	10

14 How active is your puppy?

Please circle the answer for the question above…

Not applicable	*Extremely inactive*					*Moderately active*					*Extremely*
n/a	0	1	2	3	4	5	6	7	8	9	

15 To what degree does your puppy show stiffness a†er a 'lie down'?

Please circle the answer for the question above…

Not applicable	*Not at all*					*Moderately*					*Extremely*
n/a	0	1	2	3	4	5	6	7	8	9	10

Section 4 – Mobility at exercise

16 How keen is your puppy to exercise?

Please circle the answer for the question above…

Not applicable	*Not at all*					*Moderately*					*Extremely*
n/a	0	1	2	3	4	5	6	7	8	9	10

17 How would you rate your puppy's ability to exercise?

Please circle the answer for the question above…

Not applicable	*Extremely poor*					*Moderate*					*Extremely good*
n/a	0	1	2	3	4	5	6	7	8	9	10

18 To what extent is your puppy's mobility adversely affected immediately af†er exercise?

Please circle the answer for the question above…

Not applicable	*Not at all*					*Moderately*					*Extremely*
n/a	0	1	2	3	4	5	6	7	8	9	10

19 How o†en does your puppy rest (stop/sit down) during exercise?

Please circle the answer for the question above…

Not applicable	*Not at all*					*Moderately*					*Extremely frequently*
n/a	0	1	2	3	4	5	6	7	8	9	10

---

20 To what extent does cold, damp weather reduce your puppy's ability to exercise?

Please circle the answer for the question above…

Not applicable	*Not at all*					*Moderately*					*Ability to exercise extremely reduced*
n/a	0	1	2	3	4	5	6	7	8	9	10

---

21 To what extent does stiffness reduce your puppy's ability to exercise?

Please circle the answer for the question above…

Not applicable	*Not at all*					*Moderately*					*Ability to exercise extremely reduced*
n/a	0	1	2	3	4	5	6	7	8	9	10

22 Please use the space below to add any other information about your dog that you would like to share with us…

Please use the space below to share any other information with us…

Thank you so much for your help in completing this questionnaire.
Pleaseusethefreepostenvelopeprovidedtosendthisbacktous
at your earliest convenience!

Retro-reflective marker positions for labelling anatomical locations in Qualisys

S8

Reflective Marker Number	Anatomical Landmark
1/2 (L/R)	*Frontal lobe*
3	*Occipital crest*
4/5 (L/R)	*Cervical vertebrae 1*
6	*Cervical vertebrae 7*
7/8 (L/R)	*Top of scapulae*
9/10 (L/R)	*Acromion*
11/12 (L/R)	*Mid humerus*
13/14 (L/R)	*Medial epicondyle*
15/16 (L/R)	*Lateral epicondyle*
17/18 (L/R)	*Lateral ulna styloid*
19/20 (L/R)	*Lateral metacarpophalangeal joint*
21/22 (L/R)	*Thoracic toe*
23	*Thoracic vertebrae 13*
24	*Lumbar vertebrae 7*
25/26 (L/R)	*Wing of ilium*
27/28 (L/R)	*Ischial tuberosity*
29/30 (L/R)	*Greater trochanter of the femur*
31/32 (L/R)	*Mid femur*
33/34 (L/R)	*Medial epicondyle*
35/36 (L/R)	*Lateral epicondyle*
37/38 (L/R)	*Proximal tibia*
39/40 (L/R)	*Lateral malleolus*
41/42 (L/R)	*Lateral metatarsophalangeal joint*
43/44 (L/R)	*Pelvic toe*

PID Number	PVF_LF (N/Kg)	PVF_LH (N/Kg)	PVF_RF (N/Kg)	PVF_RH (N/Kg)
1.00	1.30	0.85	1.28	0.82
2.00	0.77	0.69	0.69	0.65
3.00	1.26	0.83	1.23	0.79
4.00	1.34	0.95	1.34	0.94
5.00	1.13	0.80	1.04	0.80
6.00	1.30	0.90	1.26	0.84
7.00	0.91	0.71	0.97	0.66
8.00	1.04	0.75	1.03	0.72
9.00	1.26	0.90	1.16	0.85
10.00	1.06	0.79	1.10	0.81
11.00	1.33	0.79	1.33	0.74
12.00	2.71	1.95	2.73	1.88
13.00	4.66	3.30	3.90	3.90
14.00	1.23	0.70	1.21	0.78
15.00	1.32	0.79	1.38	0.84
16.00	1.36	0.75	1.28	0.75
17.00	0.96	0.72	0.97	0.66
18.00	0.51	0.43	0.59	0.40
19.00	0.73	0.66	0.67	0.60
20.00	1.02	0.63	1.05	0.65
21.00	0.90	0.63	0.90	0.62
22.00	0.28	0.18	0.27	0.17
23.00	0.90	0.58	0.89	0.56
24.00	1.11	0.81	0.98	0.79
26.00	1.13	0.84	1.04	0.82
27.00	2.10	1.11	2.07	1.18
28.00	1.00	0.76	0.85	0.76
29.00	0.98	0.67	1.09	0.69
30.00	1.04	0.75	1.03	0.72
31.00	0.99	0.72	0.91	0.74
32.00	0.77	0.69	0.69	0.65
33.00	1.33	0.90	1.41	0.81
35.00	2.46	1.77	2.46	1.58
37.00	1.48	0.84	1.46	0.88
38.00	1.48	0.91	1.52	0.93
39.00	1.29	0.80	1.22	0.82
40.00	0.69	0.73	0.86	0.62
41.00	2.46	1.77	2.46	1.58
42.00	1.48	0.84	1.46	0.88
43.00	0.60	0.49	0.70	0.43
44.00	1.02	0.72	1.01	0.67
45.00	1.29	0.80	1.22	0.82
46.00	0.97	0.70	0.91	0.73
47.00	0.79	0.71	0.83	0.70

PID Number	PVF_LF (N/Kg)	PVF_LH (N/Kg)	PVF_RF (N/Kg)	PVF_RH (N/Kg)
48.00	1.41	1.13	1.43	1.10
49.00	1.25	0.83	1.18	0.85
50.00	0.83	0.71	0.70	0.87
51.00	1.39	0.98	1.25	0.98
52.00	1.25	0.89	1.24	0.89
53.00	1.29	0.70	1.30	0.75
54.00	1.38	0.75	1.32	0.74
55.00	1.33	0.90	1.41	0.81
56.00	2.46	1.77	2.46	1.58
57.00	1.48	0.84	1.46	0.88
58.00	1.48	0.91	1.52	0.93
59.00	1.29	0.80	1.22	0.82
59.00	0.69	0.73	0.86	0.62
60.00	0.60	0.49	0.70	0.43
61.00	1.02	0.72	1.01	0.67
62.00	0.97	0.70	0.91	0.73

PID Number	Cycle time_FL_L Seconds (s)	Cycle time_HL_L Seconds (s)	Cycle time_FL_R Seconds (s)	Cycle time_HL_R Seconds (s)
1.00	0.39	0.40	0.39	0.40
2.00	0.45	0.45	0.45	0.45
3.00	0.38	0.38	0.38	0.38
4.00	0.53	0.53	0.53	0.53
5.00	0.52	0.52	0.52	0.53
6.00	0.41	0.41	0.41	0.41
7.00	0.49	0.49	0.49	0.49
8.00	0.48	0.46	0.48	0.46
9.00	0.42	0.42	0.43	0.42
10.00	0.47	0.47	0.48	0.48
11.00	0.46	0.47	0.46	0.46
12.00	0.42	0.41	0.41	0.42
13.00	0.44	0.43	0.43	0.43
14.00	0.34	0.34	0.34	0.34
15.00	0.36	0.37	0.36	0.37
16.00	0.33	0.34	0.33	0.34
17.00	0.50	0.52	0.50	0.50
18.00	0.48	0.46	0.48	0.46
19.00	0.51	0.53	0.51	0.52
20.00	0.49	0.48	0.49	0.48
21.00	0.49	0.48	0.48	0.48
22.00	0.40	0.41	0.41	0.40
23.00	0.50	0.51	0.50	0.51
24.00	0.47	0.47	0.47	0.46
26.00	0.46	0.46	0.46	0.46
27.00	0.55	0.53	0.55	0.54
28.00	0.43	0.43	0.42	0.43
29.00	0.48	0.47	0.48	0.48
30.00	0.49	0.41	0.51	0.48
31.00	0.51	0.51	0.51	0.51
32.00	0.47	0.47	0.47	0.46
33.00	0.39	0.40	0.39	0.39
35.00	0.51	0.49	0.51	0.49
37.00	0.39	0.39	0.38	0.39
38.00	0.47	0.47	0.47	0.47
39.00	0.40	0.40	0.40	0.40
40.00	0.42	0.42	0.42	0.42
41.00	0.47	0.47	0.47	0.47
42.00	0.39	0.40	0.39	0.39
43.00	0.43	0.41	0.42	0.41
44.00	0.53	0.54	0.54	0.54
45.00	0.39	0.40	0.39	0.39
46.00	0.51	0.50	0.50	0.51
47.00	0.47	0.47	0.47	0.47

PID Number	Cycle time_FL_L Seconds (s)	Cycle time_HL_L Seconds (s)	Cycle time_FL_R Seconds (s)	Cycle time_HL_R Seconds (s)
48.00	0.49	0.49	0.49	0.49
49.00	0.31	0.31	0.31	0.32
50.00	0.37	0.37	0.37	0.37
51.00	0.38	0.38	0.38	0.38
52.00	0.33	0.33	0.33	0.34
53.00	0.35	0.35	0.34	0.35
54.00	0.37	0.37	0.37	0.37
55.00	0.38	0.38	0.38	0.38
56.00	0.36	0.36	0.36	0.36
57.00	0.03	0.03	0.03	0.02
58.00	0.02	0.02	0.02	0.02
59.00	0.35	0.35	0.35	0.35
59.00	0.38	0.38	0.38	0.38
60.00	0.33	0.33	0.33	0.34
61.00	0.35	0.35	0.34	0.35
62.00	0.31	0.31	0.31	0.32

PID Number	Duty factor_FL_L Seconds (s)	Duty factor_HL_L Seconds (s)	Duty factor_FL_R Seconds (s)	Duty factor_HL_R Seconds (s)
1.00	0.42	0.35	0.43	0.36
2.00	0.57	0.53	0.55	0.55

3.00	0.45	0.38	0.44	0.35
4.00	0.50	0.43	0.51	0.45
5.00	0.49	0.41	0.50	0.44
6.00	0.45	0.39	0.46	0.39
7.00	0.47	0.45	0.48	0.48
8.00	0.49	0.42	0.52	0.46
9.00	0.47	0.41	0.47	0.39
10.00	0.46	0.44	0.47	0.44
11.00	0.41	0.36	0.43	0.37
12.00	0.41	0.39	0.41	0.40
13.00	0.50	0.45	0.51	0.43
14.00	0.41	0.37	0.43	0.37
15.00	0.44	0.42	0.45	0.41
16.00	0.43	0.39	0.42	0.37
17.00	0.46	0.45	0.49	0.45
18.00	0.49	0.42	0.52	0.46
19.00	0.48	0.50	0.49	0.48
20.00	0.49	0.41	0.48	0.42
21.00	0.49	0.42	0.49	0.43
22.00	0.52	0.44	0.51	0.43
23.00	0.47	0.41	0.48	0.42
24.00	0.50	0.43	0.51	0.43
26.00	0.49	0.43	0.52	0.43
27.00	0.49	0.45	0.48	0.42
28.00	0.49	0.43	0.50	0.44
29.00	0.42	0.39	0.43	0.37
30.00	0.49	0.38	0.46	0.48
31.00	0.51	0.45	0.53	0.47
32.00	0.50	0.43	0.51	0.43
33.00	0.42	0.37	0.42	0.34
35.00	0.46	0.39	0.45	0.42
37.00	0.39	0.39	0.39	0.37
38.00	0.40	0.38	0.41	0.37
39.00	0.41	0.38	0.42	0.38
40.00	0.49	0.46	0.47	0.46
41.00	0.40	0.38	0.41	0.37
42.00	0.42	0.37	0.42	0.34
43.00	0.52	0.47	0.52	0.50
44.00	0.51	0.45	0.50	0.43
45.00	0.42	0.37	0.42	0.34
46.00	0.51	0.45	0.53	0.48
47.00	0.40	0.38	0.41	0.37
PID Number	**Duty factor_FL_L Seconds (s)**	**Duty factor_HL_L Seconds (s)**	**Duty factor_FL_R Seconds (s)**	**Duty factor_HL_R Seconds (s)**
48.00	0.50	0.48	0.51	0.49
49.00	0.43	0.38	0.40	0.34

50.00	0.40	0.38	0.40	0.39
51.00	0.46	0.33	0.45	0.36
52.00	0.44	0.38	0.45	0.40
53.00	0.43	0.42	0.44	0.39
54.00	0.43	0.36	0.41	0.35
55.00	0.46	0.33	0.45	0.36
56.00	0.44	0.38	0.45	0.40
57.00	0.44	0.37	0.43	0.37
58.00	0.02	0.03	0.02	0.02
59.00	0.01	0.02	0.02	0.02
59.00	0.43	0.38	0.40	0.34
60.00	0.44	0.38	0.45	0.40
61.00	0.43	0.42	0.44	0.39
62.00	0.43	0.36	0.41	0.35

PID Number	**Stance time_FL_L Seconds (s)**	**Stance time_HL_L Seconds (s)**	**Stance time_FL_R Seconds (s)**	**Stance time_HL_R Seconds (s)**
1.00	0.17	0.14	0.17	0.14
2.00	0.26	0.23	0.24	0.25
3.00	0.17	0.14	0.17	0.13
4.00	0.26	0.23	0.27	0.24

5.00	0.25	0.22	0.26	0.24
6.00	0.18	0.16	0.19	0.16
7.00	0.23	0.22	0.23	0.24
8.00	0.24	0.20	0.25	0.21
9.00	0.20	0.18	0.20	0.17
10.00	0.22	0.21	0.22	0.21
11.00	0.19	0.17	0.20	0.17
12.00	0.18	0.16	0.17	0.17
13.00	0.22	0.20	0.23	0.19
14.00	0.14	0.13	0.14	0.13
15.00	0.16	0.15	0.16	0.15
16.00	0.15	0.13	0.14	0.13
17.00	0.23	0.23	0.25	0.23
18.00	0.24	0.20	0.25	0.21
19.00	0.24	0.25	0.24	0.24
20.00	0.24	0.20	0.24	0.21
21.00	0.24	0.20	0.24	0.21
22.00	0.21	0.18	0.20	0.17
23.00	0.24	0.21	0.24	0.23
24.00	0.24	0.20	0.24	0.21
26.00	0.43	0.20	0.24	0.20
27.00	0.27	0.24	0.27	0.23
28.00	0.21	0.19	0.22	0.19
29.00	0.20	0.18	0.20	0.18
30.00	0.25	0.18	0.22	0.23
31.00	0.26	0.24	0.28	0.24
32.00	0.24	0.25	0.24	0.24
33.00	0.17	0.15	0.17	0.13
35.00	0.23	0.19	0.23	0.20
37.00	0.15	0.14	0.15	0.14
38.00	0.19	0.18	0.19	0.18
39.00	0.16	0.15	0.17	0.15
40.00	0.21	0.19	0.20	0.20
41.00	0.19	0.18	0.19	0.18
42.00	0.17	0.15	0.17	0.13
43.00	0.21	0.18	0.21	0.20
44.00	0.28	0.24	0.27	0.24
45.00	0.17	0.15	0.17	0.13
46.00	0.26	0.23	0.27	0.25
47.00	0.19	0.18	0.19	0.18
PID Number	**Stance time_FL_L Seconds (s)**	**Stance time_HL_L Seconds (s)**	**Stance time_FL_R Seconds (s)**	**Stance time_HL_R Seconds (s)**
48.00	0.25	0.24	0.25	0.24
49.00	0.15	0.14	0.14	0.12
50.00	0.13	0.12	0.12	0.12
51.00	0.17	0.13	0.17	0.13

52.00	0.17	0.15	0.17	0.15
53.00	0.14	0.14	0.14	0.13
54.00	0.15	0.13	0.14	0.12
55.00	0.17	0.13	0.17	0.13
56.00	0.17	0.15	0.17	0.15
57.00	0.16	0.13	0.15	0.13
58.00	0.02	0.01	0.02	0.01
59.00	0.01	0.01	0.02	0.02
59.00	0.15	0.14	0.14	0.12
60.00	0.17	0.15	0.17	0.15
61.00	0.14	0.14	0.14	0.13
62.00	0.15	0.13	0.14	0.12

PID Number	**Stride length_FL_L Metres (m)**	**Stride length_HL_L Metres (m)**	**Stride length_FL_R Metres (m)**	**Stride length_HL_R Metres (m)**
1.00	0.81	0.82	0.80	0.81
2.00	0.53	0.52	0.53	0.52
3.00	0.68	0.68	0.68	0.68
4.00	0.75	0.75	0.75	0.76

5.00	0.76	0.79	0.77	0.79
6.00	0.96	0.97	0.95	1.00
7.00	0.98	0.96	0.97	0.97
8.00	0.65	0.63	0.64	0.62
9.00	1.04	1.02	1.03	1.03
10.00	1.07	1.06	1.08	1.08
11.00	1.03	1.04	1.03	1.03
12.00	0.96	0.94	0.95	0.94
13.00	1.03	1.04	1.02	1.02
14.00	0.59	0.59	0.59	0.59
15.00	0.63	0.62	0.65	0.65
16.00	0.49	0.49	0.49	0.49
17.00	0.95	0.95	0.96	0.97
18.00	0.65	0.63	0.64	0.62
19.00	0.93	0.94	0.94	0.95
20.00	1.04	1.03	1.05	1.04
21.00	0.93	0.91	0.94	0.92
22.00	0.53	0.52	0.52	0.51
23.00	0.94	0.94	0.94	0.95
24.00	0.90	0.92	0.92	0.93
26.00	0.93	0.93	0.92	0.93
27.00	1.07	1.03	1.08	1.06
28.00	1.06	1.08	1.06	1.07
29.00	1.06	1.05	1.04	1.03
30.00	0.98	0.97	0.67	0.89
31.00	0.86	0.87	0.87	0.87
32.00	0.93	0.92	0.92	0.93
33.00	0.81	0.81	0.81	0.81
35.00	1.19	1.16	1.20	1.15
37.00	0.85	0.86	0.86	0.86
38.00	0.69	0.70	0.70	0.70
39.00	0.77	0.78	0.77	0.85
40.00	0.68	0.69	0.67	0.69
41.00	0.69	0.70	0.70	0.70
42.00	0.81	0.81	0.81	0.81
43.00	0.59	0.57	0.59	0.57
44.00	0.78	0.78	0.79	0.80
45.00	0.81	0.81	0.81	0.81
46.00	0.88	0.88	0.88	0.88
47.00	0.69	0.70	0.70	0.70
PID Number	**Stride length_FL_L Metres (m)**	**Stride length_HL_L Metres (m)**	**Stride length_FL_R Metres (m)**	**Stride length_HL_R Metres (m)**
48.00	0.90	0.90	0.90	0.90
49.00	0.54	0.54	0.54	0.54
50.00	0.60	0.60	0.60	0.59
51.00	0.55	0.54	0.54	0.55

52.00	0.51	0.51	0.51	0.51
53.00	0.47	0.46	0.48	0.47
54.00	0.54	0.54	0.54	0.53
55.00	0.55	0.54	0.54	0.55
56.00	0.51	0.51	0.51	0.51
57.00	0.53	0.53	0.53	0.53
58.00	0.04	0.04	0.04	0.04
59.00	0.05	0.05	0.05	0.05
59.00	0.54	0.54	0.54	0.54
60.00	0.51	0.51	0.51	0.51
61.00	0.47	0.46	0.48	0.47
62.00	0.54	0.54	0.54	0.53

PID Number	**Swing time_FL_L Seconds (s)**	**Swing time_HL_L Seconds (s)**	**Swing time_FL_R Seconds (s)**	**Swing time_HL_R Seconds (s)**
1.00	0.23	0.26	0.23	0.26
2.00	0.19	0.21	0.20	0.20
3.00	0.21	0.23	0.21	0.24
4.00	0.26	0.30	0.26	0.29

5.00	0.27	0.31	0.26	0.29
6.00	0.22	0.25	0.22	0.25
7.00	0.26	0.27	0.26	0.26
8.00	0.25	0.26	0.23	0.25
9.00	0.22	0.25	0.22	0.26
10.00	0.25	0.26	0.25	0.27
11.00	0.27	0.30	0.26	0.29
12.00	0.24	0.25	0.24	0.25
13.00	0.22	0.24	0.21	0.25
14.00	0.20	0.22	0.19	0.21
15.00	0.20	0.21	0.20	0.21
16.00	0.19	0.21	0.19	0.21
17.00	0.27	0.28	0.25	0.28
18.00	0.25	0.26	0.23	0.25
19.00	0.27	0.26	0.26	0.27
20.00	0.25	0.28	0.25	0.27
21.00	0.25	0.27	0.25	0.27
22.00	0.19	0.22	0.20	0.21
23.00	0.26	0.30	0.26	0.29
24.00	0.23	0.27	0.23	0.26
26.00	0.23	0.26	0.22	0.26
27.00	0.28	0.30	0.28	0.31
28.00	0.22	0.24	0.21	0.24
29.00	0.28	0.29	0.27	0.30
30.00	0.26	0.24	0.22	0.26
31.00	0.25	0.28	0.24	0.27
32.00	0.23	0.27	0.21	0.24
33.00	0.23	0.25	0.23	0.26
35.00	0.27	0.30	0.28	0.28
37.00	0.24	0.24	0.23	0.24
38.00	0.28	0.29	0.27	0.30
39.00	0.23	0.24	0.23	0.25
40.00	0.21	0.23	0.22	0.23
41.00	0.28	0.29	0.27	0.30
42.00	0.23	0.25	0.23	0.26
43.00	0.21	0.22	0.20	0.21
44.00	0.26	0.29	0.27	0.31
45.00	0.23	0.25	0.23	0.26
46.00	0.25	0.27	0.23	0.26
47.00	0.28	0.29	0.27	0.30
PID Number	**Swing time_FL_L Seconds (s)**	**Swing time_HL_L Seconds (s)**	**Swing time_FL_R Seconds (s)**	**Swing time_HL_R Seconds (s)**
48.00	0.24	0.25	0.24	0.25
49.00	0.20	0.22	0.21	0.23
50.00	0.19	0.20	0.19	0.19
51.00	0.20	0.24	0.20	0.24

52.00	0.21	0.24	0.21	0.23
53.00	0.19	0.19	0.18	0.20
54.00	0.20	0.22	0.20	0.23
55.00	0.20	0.24	0.20	0.24
56.00	0.21	0.24	0.21	0.23
57.00	0.20	0.22	0.20	0.22
58.00	0.01	0.02	0.01	0.02
59.00	0.01	0.01	0.01	0.01
59.00	0.20	0.22	0.21	0.23
60.00	0.21	0.24	0.21	0.23
61.00	0.19	0.19	0.18	0.20
62.00	0.20	0.22	0.20	0.23

PID Number	Step width FL Metres (m)	Step width HL Metres (m)	Average Speed (seconds/s)
1.00	0.09	0.08	1.74
2.00	0.15	0.14	1.06
3.00	0.17	0.15	1.70
4.00	0.14	0.12	1.03

5.00	0.11	0.12	0.95
6.00	0.14	0.14	2.06
7.00	0.18	0.07	1.92
8.00	0.14	0.13	1.20
9.00	0.11	0.10	2.20
10.00	0.15	0.15	1.78
11.00	0.14	0.12	1.83
12.00	0.13	0.07	2.05
13.00	0.19	0.14	1.98
14.00	0.13	0.14	1.51
15.00	0.10	0.13	1.22
16.00	0.10	0.10	1.40
17.00	0.21	0.14	1.87
18.00	0.14	0.13	1.20
19.00	0.13	0.16	1.73
20.00	0.16	0.12	1.86
21.00	0.12	0.13	1.73
22.00	0.12	0.12	1.35
23.00	0.16	0.15	1.76
24.00	0.20	0.16	1.93
26.00	0.16	0.16	1.94
27.00	0.18	0.18	1.54
28.00	0.19	0.14	2.08
29.00	0.22	0.14	1.81
30.00	0.18	0.14	1.87
31.00	0.20	0.18	1.68
32.00	0.13	0.16	2.08
33.00	0.08	0.11	1.86
35.00	0.18	0.25	2.06
37.00	0.13	0.09	1.50
38.00	0.17	0.09	1.24
39.00	0.12	0.15	1.55
40.00	0.13	0.08	1.63
41.00	0.17	0.09	1.24
42.00	0.08	0.11	1.86
43.00	0.14	0.14	1.37
44.00	0.13	0.14	1.33
45.00	0.08	0.11	1.86
46.00	0.18	0.16	1.71
47.00	0.17	0.09	1.24
PID Number	**Step width FL Metres (m)**	**Step width HL Metres (m)**	**Average Speed (seconds/s)**
48.00	0.18	0.17	1.80
49.00	0.10	0.10	1.18
50.00	0.18	0.15	1.69
51.00	0.19	0.10	1.05

52.00	0.13	0.10	1.04
53.00	0.08	0.07	1.40
54.00	0.11	0.11	1.52
55.00	0.19	0.10	1.05
56.00	0.13	0.10	1.04
57.00	0.14	0.10	1.25
58.00	0.04	0.02	0.27
59.00	0.02	0.01	0.12
59.00	0.10	0.10	1.18
60.00	0.13	0.10	1.04
61.00	0.08	0.07	1.40
62.00	0.11	0.11	1.52