Regenerative medicine for treatment of canine elbow osteoarthritis

A Thesis Submitted by

Simone Anesi DVM DipECVS MRCVS

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School of Veterinary Medicine, College of Medical, Veterinary & Life Sciences University of Glasgow

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1 INTRODUCTION

1.1 ABSTRACT

This masters project explores the use of two regenerative therapies (i.e V-PET platelet concentrate and Orthokine autologous conditioned sera) as treatment for dogs suffering from elbow osteoarthritis. Cases were recruited from patients presented to the orthopaedic service of the Small Animal Hospital of the University of Glasgow, and also directly from the general veterinary practices in the area. Nine dogs met the inclusion criteria: eight were given either V-Pet (three dogs) or Orthokine (five dogs) administered intraarticularly into the worse affected elbow, and one dog was given both treatments, one year apart. No patient suffered any adverse side effects.

Response to treatment was assessed using a pressure walkway (Strideway HRSW3, Tekscan, South Boston, USA), a LOAD questionnaire and a VonFrey electronic anaesthesiometer.

No statistically significant differences were identified in outcomes between the two treatment groups, but results were very likely affected by the small size of the population studied (potential for Type 2 error).

Based on the results of this project it was not possible to provide evidence of an effect of these therapies on the clinical signs of elbow osteoarthritis in dogs, and further research in a larger population is warranted. However, the treatments were well tolerated with no reported side effects.

1.2 PATHOGENESIS OF, AND TREATMENT OPTIONS FOR, ELBOW OSTEOARTHRITIS (OA)

1.2.1 Background

Elbow diseases are a frequent cause of lameness in both young (Demko and McLaughlin, 2005) and older dogs (Mielke *et al.*, 2018). Several different diseases can affect the canine elbow, the most common being developmental elbow disease, also named elbow dysplasia (Michelsen, 2013). Other reported forms of elbow pathology include humeral intracondylar fissures (Moores and Moores, 2017; Marcellin-Little *et al.*, 1994), luxation, septic arthritis (Mielke *et al.*, 2018) and fractures.

Each of these problems usually leads to similar clinical signs which include lameness, pain, and subsequent development of osteoarthritis, with a significant negative effect on patients' welfare (Demko and McLaughlin, 2005).

"Elbow dysplasia" is an umbrella term which includes a group of different conditions. When this term was first reported in 1965 it included elbow osteoarthrosis with or without ununited anconeal process which was thought to originate from abnormal elbow joint development (Corley and Carlson, 1965). Whether the different pathologic lesions usually encountered in dysplastic elbows share the same aetiology is the subject of ongoing debate making the term elbow dysplasia possibly not ideal. For this reason, recently, "developmental elbow disease" was proposed as a more appropriate umbrella term to be used instead of elbow dysplasia. At present there is still disagreement about which pathologies of the elbow should be included, with current inconsistent inclusion of elbow incongruity and ununited medial epicondyle (Fitzpatrick *et al.*, 2009). The three conditions that are consistently included are medial coronoid disease, ununited anconeal process and osteochondrosis of the humeral condyle (Michelsen, 2013; Keller *et al.*, 1997).

Developmental elbow disease has been reported in 17% of Labrador Retrievers in the United States, and in 70% of Bernese Mountain Dogs in the Netherlands. Large breed dogs and males appear to be over-represented, and increased risk has also been noted in some chondrodystrophic breeds such as French Bulldogs and Dachshunds (Michelsen, 2013; Meyer-Lindenberg, Fehr and Nolte, 2006). Strong evidence of a genetic predisposition has been reported in some dog breeds including Rottweilers, German Shepherd Dogs, Bernese Mountain Dogs and Labrador Retrievers (Lewis *et al.*, 2011).

Developmental elbow disease presents in with two age related peaks: young dogs at 4-12 months of age, and then older dogs of around 8 years, as OA develops (Demko and McLaughlin, 2005; Michelsen, 2013).

Depending on the specific condition affecting the elbow, both surgical and non-surgical treatments can be considered. The aim of treatment is to improve comfort levels and limb function. Irrespective of the treatment option chosen however, development of secondary osteoarthritis is frequent (Demko and McLaughlin, 2005; Michelsen, 2013).

Osteoarthritis (OA) is a chronic degenerative condition of synovial joints. It starts with an inflammatory process which causes slow and progressive damage to the cartilage and subchondral bone, with formation of osteophytes, thickening of the joint capsule, and synovitis (Malek *et al.*, 2012; Glyn-Jones *et al.*, 2015). This leads to significant pain and associated lameness (Brown *et al.*, 2007; Schaible, 2012).

At present, there is no effective therapy (medical or surgical) that will cure OA, or even completely resolve the clinical signs in all cases (Bland, 2015). Therefore, management focuses mostly on pain control by using medications such as nonsteroidal anti-inflammatory drugs, opioid analgesics, gabapentin or amantadine (Malek *et al.*, 2012; KuKanich, 2013), and weight management. Weight loss has been shown to have a positive effect on the control of clinical signs of lameness in dogs with hip OA (Impellizeri, Tetrick and Muir, 2000).

There is weak evidence that nutraceuticals such as chondroitin/glucosamine sulfate (McCarthy *et al.*, 2007) omega-3 fish oil fatty acids (Fritsch *et al.*, 2010) and beta-1,3/1,6-glucans (Beynen and Legerstee, 2010) can be administered as oral supplements to reduce clinical signs of OA and to reduce progression of the degenerative process. Intraarticular injections of hyaluronan (Brandt, Smith and Myers, 2004) and intramuscular administration of polysulfated glycosaminoglycan (Fujiki *et al.*, 2007) are other options reported for the management of OA. Acupuncture also is reported to have a positive effect as a part of a multimodal plan in pain management in veterinary patients (Stordalen *et al.*, 2020). Finally, physical rehabilitation is also adopted for the improvement of joint function and delaying of the joint's degenerative processes (Alvarez *et al.*, 2016).

While total joint replacement is often utilised with good results for management of end stage hip OA in dogs, total elbow joint replacement is rarely performed due to the high risk of catastrophic complications and unpredictable outcome. Effective treatment of OA therefore remains elusive, and over the last years, has become a major focus in the field of regenerative medicine - the field of research that focus on repair, replacement or regeneration of cells, tissues, and organs aiming to restore their structure and function (Greenwood *et al.*, 2006).

Regenerative Medicine has evolved rapidly during recent years with new therapies on based stem cells, tissue engineering, gene therapy, and usage of autologous blood product such as platelet rich plasma (PRP) or autologous conditioned sera (ACS). Platelet-rich plasma (PRP) is one of the first-known regenerative approaches proposed in clinical practice (Coppi, 2012; Guercio *et al.*, 2012; Gato-Calvo *et al.*, 2019).

In the absence of effective treatment options that can cure osteoarthritis or even reliably mitigate the clinical signs, regenerative medicine holds exciting potential for delivering treatment options in both human and veterinary medicine. This Master's project focuses on acquiring clinical data to assess the efficacy of a well-established form of regenerative medicine (platelet concentrate) and on a less established but promising form, autologous conditioned sera.

1.2.2 Platelet Concentrate Products

The importance of platelets in haemostasis is well documented: platelets are necessary for primary coagulation and also initiate the secondary coagulation process. Platelet cytoplasm contains a high number of active molecules, including extracellular matrix proteins, vasoactive peptides, cytokines and growth factors. These are released when platelets are activated and are known to play an important role in wound healing (Anitua *et al.*, 2004; Foster *et al.*, 2009; Harrison and Cramer, 1993; Qureshi *et al.*, 2009; Senzel, Gnatenko and Bahou, 2009). For example molecules such as platelet derived growth factor (PDGF), endothelial growth factor(EGF), connective tissue growth factor (CTGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) are essential for healing of both bone and soft tissues (Anitua *et al.*, 2004; Andia, Sánchez and Maffulli, 2012; Foster *et al.*, 2009; Mehta and Watson, 2008). Platelets also contain substances with antimycotic and antibacterial effects, glycoproteins that influence inflammation, ADP, ATP, calcium, histamine, serotonin, dopamine in addition to about 800 other unique molecules. (Fortier, Hackett and Cole, 2011; Qureshi *et al.*, 2009; Senzel, Gnatenko and Bahou, 2009). The large variety of active molecules increases the challenge of completely understanding the roles of platelets and platelet-derived products.

As these molecules were progressively discovered and studied over recent decades the idea of using platelet-derived products in regenerative medicine was introduced. Several terms were created to describe different platelet-based products including platelet-rich plasma, platelet-rich fibrin, platelet gel, platelet-rich concentrate, platelet concentrate and 'plasma rich in platelets'. Although any sample of plasma containing a high concentration of platelets could be defined as platelet rich plasma, the consensus is that the concentration of platelets should be at least three to five times higher than is present in peripheral blood (Kevy and Jacobson, 2004; Marx, 2001).

Depending on the technique used to isolate and concentrate the platelets there is also the potential to concurrently concentrate leukocytes generating what is defined as "leukocyte and platelet rich plasma" (L-PRP). The effects of leukocytes in the regenerative process remain unclear and controversial. Some evidence suggests that the increased concentration of VEGF and PDGF within leukocytes would assist wound healing and platelet activation (Andia, Sánchez and Maffulli, 2012). Conversely other authors suggest that the presence of leukocytes and in particular neutrophils leads to excessive inflammation and tissue damage via the release of radical oxygen species, catabolic cytokines and metalloproteinases (Sundman, Cole and Fortier, 2011).

Platelet rich plasma (PRP) should not be considered like a drug with a standardised structure and mechanism of action but rather it is an incompletely characterized blood product with expected variability among patients and depending on preparation methods. Several factors can potentially contribute to variability of PRP and these include patient variability (e.g. PCV and platelet count) concurrent drug therapy, coagulation state, sampling technique, isolation method and degree of platelet activation. It has been shown that multiple isolation techniques used on the same sample will produce PRP with different characteristics (Boswell *et al.*, 2012; Carr *et al.*, 2016; Castillo *et al.*, 2011; Franklin, Garner and Cook, 2015). Platelet rich plasma variability has also been noted when the same isolation method is used on different blood samples (Boswell *et al.*, 2012). The unpredictable variability of PRP makes interpretation of the available evidence more challenging.

1.2.2.1 Methodologies for preparation of PRP

Two main methods of preparing PRP have been described: centrifugation and filtration.

With the **centrifugation method**, blood is collected in anticoagulant and centrifuged at low speed; specific indications about time and speed depend on the kit used. The spin produces 3 layers which, from top to bottom, include: plasma, platelets and white blood cells (together forming the buffy coat) and red blood cells. The plasma and only the uppermost part of the buffy coat are collected and transferred to a new tube and centrifuged again at a higher speed for a longer time. This generates a layer of platelet-poor plasma above a layer of buffy coat rich in platelets. Most of the plasma is discarded and the buffy coat then remains suspended in a small amount of plasma resulting in a small volume of platelet rich plasma.

Alternatively, it is possible to transfer the whole buffy coat containing platelets and a high number of white blood cells into a new tube (instead of the uppermost part alone containing mostly platelets as described above), producing a plasma rich in both platelets and leukocytes.

The amount of blood that can be processed, number of spins, centrifugation speed and time, how the layer containing the platelet is selected and volume of the final product varies between kits, although the overall process is usually completed in 30-60 minutes.

The centrifugation parameters in particular need to be carefully considered as high centrifugation speeds can damage and activate the platelets leading to release of their active molecules into the plasma that is subsequently discarded, diminishing the total growth factor concentration (Gonshor,

2002). The effects of spin speed and time on canine or feline platelets have not been extensively described however. Furthermore, because platelet characteristics (mass, number, volume) vary among species it is possible that protocols developed for human medicine may not be ideal for veterinary patients (Boudreaux and Ebbe, 1998).

A **gravity driven filtration system** can also be used to generate PRP. In this system anticoagulated blood is placed into a bag and mixed with sterile water, which causes the platelets to swell. The bag is then hung, and gravity causes the blood to drip through a filter, which captures the platelets and leukocytes. Sterile saline is then flushed back in the opposite direction displacing the cells from the filter, obtaining platelets and white blood cells concentrated in saline solution. Advantages include the low cost of the equipment, and less time is needed to prepare the product. It is important to note that cells are suspended in saline rather than plasma however, eliminating any potential beneficial effect of plasma-related molecules. This system has been tested in dogs with osteoarthritis and horses with suspensory branch injury with encouraging results (Fahie *et al.*, 2013; Castelijns *et al.*, 2011). Nevertheless two studies have shown that PRP produced by filtration contains a higher number of leukocytes and erythrocytes and a lower number of platelets compared with other methods (Carr *et al.*, 2016; Franklin, Garner and Cook, 2015).

In human medicine PRP is often activated using thrombin, calcium chloride or collagen type I prior application (Foster *et al.*, 2009). Activation leads to release of the alpha granules containing the active molecules from the platelets after 10 minutes with about 95% of the total content released within an hour. Additional granules continue to be produced and released over the following days (Marx, 2004).

With the exception of two PRP systems (Protec PRP and MediVet PRP) which can produce a platelet-rich fibrin gel, all the other available products do not include an activator (Visser *et al.*, 2010). The rational for using non-activated PRP is that platelets will be activated by contact with injured tissues, extracellular matrix, and surgical blood clots.

Several point of care PRP production systems specifically designed for veterinary patients are commercially available.

- CRT Pure PRP, Canine Regenerative Therapies, FortMeyers, FL, USA;
- MediVet PRP, Medivet Biologics LLC, Nicholasville, KY, USA.
- Protec PRP, Pulse Veterinary Technologies, LLC, Alpharetta, GA, USA

- V-PET, Pall Corporation, Port Washington, NY, USA.

With exception of the V-PET system (previously named C-PET or E-PET) which is based on gravity and filtration all the other systems are based on centrifugation.

1.2.2.2 Review of literature on use of PRP in human patients.

1.2.2.2.1 USE OF PRP IN THE TREATMENT OF TENDINOPATHIES

PRP increases the proliferation of mesenchymal stem cells and fibroblasts within tendons and also the production of collagen type I within tendon fibroblasts (Rodríguez-Jiménez *et al.*, 2012; Klein *et al.*, 2002). In rats with calcaneal tendon defects, injection of PRP into the lesion resulted in increased tendon strength and stiffness compared to a control group (Asperben and Virchenko, 2004). Also, injection of PRP into a rat patellar tendon injury model resulted in increased collagen production within the injury site (Kajikawa *et al.*, 2008).

In human medicine PRP has been described as a possible treatment for calcaneal tendinopathy, patellar tendinopathy and lateral epicondylitis, with several studies reporting encouraging results (Alfredson and Lorentzon, 2000; Dragoo *et al.*, 2014; Mishra and Pavelko, 2006; Sánchez *et al.*, 2007; Vetrano *et al.*, 2013).

Liddle et al. (2015) evaluated eleven studies on the use of PRP in the treatment of patellar tendinopathy in people. Two of these were randomized double blinded clinical trials. The authors concluded that PRP is a safe and promising therapy in the treatment of recalcitrant patellar tendinopathy. However, its superiority over other treatments such as physical therapy remained unproven.

Despite this, several systematic reviews failed to identify a clear positive clinical effect of PRP in the treatment of tendinopathies in people. A systematic review by De Vos, Windt and Weir (2014) evaluating the effect of PRP on chronic lateral epicondylar tendinopathy found strong evidence to suggest that PRP is not efficacious in the management of this disease in people. Di Matteo *et al.* (2015) systematically reviewed the literature in human medicine on the efficacy of PRP in the treatment of patellar and Achilles tendinopathy and identified 22 studies. Of these, only two were double blinded randomized controlled clinical trials (RCT's) (one on Achilles tendinopathy and one on patellar tendinopathy). Considering patellar tendinopathy, all the reports suggested a favourable role for PRP in stimulating tendon healing and providing symptomatic relief. Results were more controversial with

Achilles tendinopathy, since the only double-blind RCT showed no beneficial effect for PRP, whereas the remaining studies (all case series) reported overall positive outcomes even at mid/long-term evaluation. The main finding of their study was that there is a paucity of high-level evidence regarding the use of PRP in the management of tendinopathy (both patellar and Achilles), making it difficult to draw any conclusions about the efficacy of such treatment in managing these conditions.

Similarly, Figueroa *et al.*, (2015) evaluated the available evidence for the use of PRP to augment anterior cruciate ligament repair surgery in humans. Only randomized controlled trials or prospective cohort studies were included. Authors concluded that concerning ACL graft maturation, there was promising evidence that the addition of PRP could be a synergic factor in acquiring maturity more quickly than grafts with no PRP, with the clinical implication of this remaining unclear. There was no proof that clinical outcomes of ACL surgery were enhanced by the use of PRP.

1.2.2.2.2 USE OF PRP IN THE TREATMENT OF BONE DEFECTS

Platelets contain several growth factors that are involved with long bone growth and remodelling including PDGF, TGF-β, VEGF and bone morphogenic proteins (BMP's). Although there is some evidence that PRP can be used to augment bone graft efficacy when treating aseptic non-unions (Sanchez *et al.*, 2009), there is no evidence that if used alone PRP could accelerate cortical bone healing.

Several systematic reviews failed to clearly identify a positive clinical effect. Griffin *et al.* (2009) published a literature review in 2008 analysing evidence for the use of PRP in augmenting bone healing. Only five studies met their inclusion criteria, of which only one was a randomized clinical trial; this was underpowered because of the outcome measures adopted. The authors concluded that the use of platelet-rich plasma was safe and feasible, but that there was no clinical evidence of benefit in either normal or delayed fracture healing.

A more recent systematic review by Lemos *et al.*, (2016) evaluated the effect of combining platelet-rich plasma (PRP) with bone grafts on bone formation and implant survival in maxillary augmentation. After inclusion and exclusion criteria were applied, 17 studies were selected for qualitative analysis and 13 for quantitative analysis. A total of 369 patients and 621 maxillary sinus augmentations were evaluated. The results showed no significant difference in implant stability or bone formation, leading the authors to conclude that there is no influence of PRP with bone graft on bone formation and implant survival in maxillary sinus augmentation.

Pocaterra *et al.*, (2016) assessed the evidence on the effectiveness of PRP as an adjunctive material in the sinus floor elevation technique in people. Only randomized controlled clinical trials comparing a group receiving PRP as an adjunctive material to a control group without PRP, involving adult human subjects with no systemic disease, were included. Of the studies identified, only one reported a significant difference in bone augmentation in favour of the adjunctive use of PRP, while four studies did not find any significant difference. None of the studies included reported a significant difference in the implant survival rate. The authors concluded that evidence available is insufficient and further randomized clinical trials are needed to clarify the effectiveness of adjunctive PRP.

1.2.2.2.3 USE OF PRP IN THE TREATMENT OF OSTEOARTHRITIS AND CARTILAGE DEFECTS

PRP has a potential role in treating cartilage disease and defects. An in vitro study showed platelet lysate caused a significant increase in porcine chondrocyte proliferation and accumulation of glycosaminoglycans and type II collagen (Akeda *et al.*, 2006). PRP induced the expression of proteins related to chondrogenic differentiation such as aggrecans, SOX-9 and COL2, in human chondrocyte cultures (Spreafico *et al.*, 2009). PRP does inhibit the expression of inflammatory mediators involved in the osteoarthritic process such as COX-2 and factor ĸB (Bendinelli *et al.*, 2010). Also in the treatment of experimentally induced cartilage lesions in rabbits, sheep and dogs, groups treated with intraarticular PRP had more complete healing (Milano *et al.*, 2010; Sun *et al.*, 2010; Kazemi and Fakhrjou, 2015). PRP has been used in human medicine to treat acute cartilage injury and osteoarthritis with promising results, although the quality of the evidence was low due to the retrospective nature of the study (Sanchez *et al.*, 2008).

Brossi *et al.*, (2015) published a systematic review of the literature on the efficacy of platelet rich plasma (PRP) in treating tendon, ligament or articular lesions in equine and human patients. One hundred and twentythree studies were included, involving randomized trials, cohort clinical studies and case series with a control group. In addition, experimental studies relevant to the clarification of PRP's effects and mechanisms of action in tissues of interest, conducted in any animal species, were selected. The authors report that beneficial effects of PRP were reported in 46.7% of the clinical studies, while no benefits were reported in 43.3%. Of the experimental studies, 73% yielded positive results, and 7.9% reported no benefits. The most frequent flaws in the design of the clinical trials were the lack of a true placebo group, poor product characterization, insufficient blinding, small sample sizes, short follow-up periods, and use of poor outcome measures. In particular, the methods of PRP preparation and administration and the selected outcome measures varied greatly between studies.

Poor study design was a common feature of equine clinical trials in particular. Of the studies in which PRP had beneficial effects, 67.8% had an overall high risk of bias. Of the studies in which PRP failed to exhibit beneficial effects, 67.8% had an overall low risk of bias. In general, although the majority of equine clinical studies reported positive results, the human clinical trials did not. In both species, positive results were more frequently observed in studies with a high risk of bias. The authors concluded that the use of PRP in musculoskeletal lesions, although safe and promising, had not shown strong evidence in clinical scenarios.

In the same year, two more targeted reviews were published looking at the efficacy of PRP in treating degenerative joint disease in human knees. Campbell *et al.*, (2015) performed a systematic review of meta-analyses evaluating PRP injection in the treatment of knee joint cartilage degenerative pathology in humans. Literature searches were performed for meta-analyses examining the use of PRP versus corticosteroids, hyaluronic acid, oral nonsteroidal anti-inflammatory drugs, or placebo. Three high quality meta-analyses met the eligibility criteria and compared outcomes of treatment with intra-articular PRP versus control (intra-articular hyaluronic acid or intra-articular placebo). The three meta-analyses were concordant and high-quality, and all showed that PRP produced clinically relevant improvements in function and reduced pain compared with the control treatment, particularly in patients with early radiographic signs of degenerative changes. Use of PRP led to significant improvements in patient outcomes at 6 months after injection; the improvements started at 2 months and were maintained for up to 12 months. However, it was unclear whether the use of multiple PRP injections led to better outcomes, and in fact the use of multiple PRP injections may increase the risk of self-limiting local adverse reactions. The authors concluded that Intra articular PRP is a viable treatment for knee OA and has the potential to lead to symptomatic relief for up to 12 months.

Lai *et al.* (2015) also analysed the literature on the efficacy of intra-articular injections of PRP for treatment of knee osteoarthritis in humans. Eight prospective clinical studies (clinical trials and observational studies) were included, all of which were published between 2010 and 2013. Half of the studies were prospective observational studies that included only PRP treatment; the rest were prospective comparative studies including both PRP and controls and 2 were randomized controlled trials. Of the 4 comparative studies, 3 compared PRP with hyaluronic acid, considered a commonly used effective treatment for knee OA; the other one used saline injection (i.e. placebo) as the control. Although most of the analyses involved small sample sizes and as a result were inconclusive, the findings consistently indicated that PRP might improve outcomes in younger patients and those with less degeneration. The authors concluded that intra-articular injections of PRP into the knee *may* have

potential as an alternative treatment for knee OA, but advised that large, multicentric randomized trial studies were needed to further assess this.

Interestingly, a review on the intra-articular use of PRP for knee OA published in the following year by Meheux *et al.* (2016) considered only publications with level I evidence, and identified six articles (739 patients, 817 knees), in which all reported significant improvements in statistical and clinical outcomes, including pain, physical function, and stiffness, with PRP. All but one study showed significant differences in clinical outcomes between PRP and hyaluronic acid (HA) or PRP and placebo in pain and function. Mean post-treatment Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores for PRP were significantly better than for HA at 3 to 6 months and at 6 to 12 months. None of the included studies used corticosteroids. The authors concluded that in patients with symptomatic knee OA, PRP injection results in significant clinical improvement for up to 12 months post injection. Clinical outcomes and WOMAC scores were significantly better after PRP versus HA at 3 to 12 months post injection.

Only one systematic review was identified that involved the use of PRP and a joint other than the knee. Vannini *et al.* (2015) performed a systematic review of the clinical literature on the use of PRP to treat ankle cartilage disorders in humans and identified 7 papers. PRP was used in two different ways: 5 of the available papers focus on its use to augment various surgical techniques for cartilage regeneration, while only two studies report its application through intra-articular injections. The authors concluded that, based on the limited number of clinical studies available, there were few major adverse events related to PRP and overall good results for the treatment of ankle cartilage pathology. Authors specified that further high-quality clinical trials in the ankle are still required to identify how PRP might be most effectively used.

1.2.2.3 Review of veterinary literature on PRP

Compared to the literature available in human medicine only a limited number of studies have described characteristics and effects of PRP in dogs. The structure of these studies varies significantly as some aimed only to describe and validate the use of PRP systems on canine blood, while other described the effects of PRP. In the second group, the majority are experimental studies that describe the effects of PRP on experimentally induced injuries, while only four studies describe clinical application and outcome of PRP on client owned dogs with naturally occurring disease.

1.2.2.3.1 DESCRIPTIVE STUDIES

Five studies have described commercially available PRP preparation kits and related products (Thoesen *et al.*, 2006; Stief *et al.*, 2011; Franklin *et al*., 2015; Carr *et al.*, 2016; Frye *et al.*, 2016).

In the studies of Carr et al. (2016) and Franklin *et al.,* (2015), PRP was produced from the same patients using different kits and products were compared

In the work of Franklin et al. (2015) each of five commercially available systems were used on blood samples from fifteen dogs and the final products were compared in respect of platelet, leukocytes and red blood cells content. Four of the systems evaluated were centrifugation based: 1. Protec PRP, PulseVet; 2. MediVetPRP, medivet America; 4. SmartPReP2, harvest technologies; 5. Angel, Arthrex Vet Systems, while one was filtration based (3. C-PET, Pall Corporation). The final products differed substantially in all the parameters evaluated when compared to the original blood sample from which they were derived. All but one system (System 1) produced a product with an increased platelet concentration, and the products from systems 3 and 4 also had significantly higher WBC concentrations.

Similarly, Carr *et al.*, (2016) compared five systems using each system on 10 different dogs, testing 50 patients in total. The systems under test were: 1. SmartPReP2, Harvest Technologies; 2. Arthrex ACP, Arthrex; 3. CRT Pure PRP, Canine Regenerative Therapies; 4. ProTec PRP, Pulse Veterinary Technologies; 5. C-PET, Pall Corporation.

As in Franklin *et al* (2015), significant differences in platelet, leukocyte and erythrocyte concentrations were noted between systems. Three systems were tested in both studies and cellular concentrations were similar across the two studies providing some evidence that when the same system is used the final product characteristics may have a degree of consistency.

1.2.2.3.2 CLINICAL TRIALS

To the authors knowledge, only four studies have evaluated the efficacy of intra-articular administration of PRP in client owned dogs with naturally occurring disease.

In a study by Franklin and Cook (2013), ten dogs with bilateral elbow osteoarthritis were randomly divided in two groups and treated with either one intra-articular injection of PRP or an intra-articular injection of a combination of hyaluronic acid and steroids. Outcomes were evaluated via validated questionnaires and subjective blinded lameness assessment. Dogs in both groups improved over time

and although some owner questionnaires had higher (better) scores in the PRP group, this is a subjective measure, and no statistical difference was found between the two groups. Results should therefore be interpreted with caution.

In the second study, ten dogs with cranial cruciate ligament (CCL) rupture that underwent arthroscopic replacement of the ligament with fascia lata, were treated with either three intra-articular injections of PRP two weeks apart or with an oral nutraceutical (Silva, Carmona and Rezende, 2013). Gait analysis, clinical examination and radiographs were repeated at monthly intervals for 3 months. No difference was noted in the radiographic scores, but at 3 months dogs that received PRP had higher clinical scores and higher force plate metrics indicating improvement.

In the third study by Fahie *et al.* (2013) twenty dogs with clinical signs of osteoarthritis in a single joint were treated with one intra-articular injection of either PRP or saline. This study was performed in two different centres and only half of the patients had plate force analysis. All patients were evaluated via owner assigned lameness scores and pain scores. Three months after treatment no improvement was noted in any of the parameters for the control group while a significant improvement was noted in all the parameters for the PRP group.

In the fourth study, ten dogs with chronic CCL rupture were treated without surgery with a single injection of PRP and their progress monitored by mean of a pressure sensitive walkway. The authors reported improvement of symmetry index but no significant change of peak vertical forces (PVF) or vertical impulse (VI) (Venator *et al.*, 2020).

To the authors knowledge only two studies are available in the veterinary literature describing the use of PRP for the treatment of tendinopathies.

A case series with 10 dogs reported the use of ultrasound guided injection of PRP for the treatment of supraspinatus tendinopathy (Ho *et al.*, 2015). Although 6 weeks after treatment ultrasonographic features of the tendons improved in 6 patients, and 4 patients had subjective improvement of lameness reported by the owner, there was no objective improvement based on kinetic gait assessment. Results from this study should be interpreted with caution because of the lack of a control group and the risk of bias in the owner and veterinary assessment.

A case series of eleven horses with suspensory branch injury treated with local injection of PRP reported complete resolution of ultrasonographic lesions within 3 months in all treated animals and complete resolution of lameness in five animals. However, the results needs to be interpreted with caution due to the absence of a control group (Castelijns *et al.*, 2011).

There are no available studies in client owned dogs to evaluate the effect of PRP in augmenting healing of fractures or osteotomies.

There are no studies describing the clinical use of PRP in feline patients although two studies describe the characteristics of feline PRP (Silva *et al.*, 2012; Silva, Jorge U. Carmona and Rezende, 2013).

1.2.2.3.3 Experimental studies on animal models

A review by Sermer *et al* (2015) focusing on PRP enhanced scaffolds for cartilage lesion repair in animals included 14 studies. There was great variability in the method of PRP preparation, choice of scaffold, and cell source between studies. Ten reported positive effects with PRP whereas only 2 showed negative overall effects. The remaining 2 studies reported no significant differences with the use of PRP. In eleven of the twelve studies that assessed this, the gross appearance and histologic analysis of repair cartilage was improved with the addition of PRP, or no difference was seen, compared with controls. The authors concluded that PRP-augmented scaffolds have been shown to be beneficial in the articular cartilage repair process in animals based on macroscopic, histologic, and biochemical analysis. Direct comparison between studies is difficult however, due to the great variability in PRP preparation and administration (Sermer *et al.*, 2015).

In a review of experimental studies on PRP as augmentation for bone defect healing on animal models, 29 articles were included (Gianakos *et al.*, 2015). These included studies on rabbits, rats, dogs, sheep and pigs. Eighty-nine percent of studies reported significant improvement in early bone healing on histologic assessment, with eighty percent of studies reporting a significant increase in bone area on microcomputed tomography. All studies reported a significant increase in bone formation on radiographs of animals given PRP, and a higher torsional stiffness for the PRP-treated defects. Thus, in the in vivo studies evaluated, PRP confers several beneficial effects on animal long-bone models.

1.2.2.4 Limiting factors to clinical application

Current limitations that prevent recommendations for the wider use of PRP in veterinary medicine include lack of quality evidence, variability of the final product depending on the donor characteristics and system used, the lack of 'quality control' of the PRP product prior to administration and finally and inconsistency of administration protocols. One way to address the lack of quality control would be to consistently check the concentration of platelet in the final product prior to administration submitting a sample mixed with EDTA for cell count. It has been suggested that EDTA is likely to be more effective in preventing clumping than citrate, allowing a more accurate count (Mylonakis *et al.*,

2008; Prins, van Leeuwen and Teske, 2009; Stokol and Erb, 2007). In addition, healing of bone and soft tissue is complex, involving many populations of cells and an unknown number of cytokines and growth factors. Therefore, the ideal number of platelets to be delivered, the ideal concentration of growth factors within PRP and proper delivery timelines have not been established. Reported administration protocols are inconsistent, a number of papers in the literature report results after one single administration (Fahie *et al.*, 2013; Murray *et al.*, 2006; Rabillard *et al.*, 2009; Souza *et al.*, 2012). Other have used several administrations (Silva, Carmona and Rezende, 2013). Inconsistent reported administration timing together with variable characteristics of the final product makes challenging to identify ideal dose timing from the existing literature.

Although several studies have described positive outcome in both veterinary and human medicine a significant number of these have low sample sizes, are not controlled, or are biased. When taking into consideration only outcomes of randomised, placebo controlled, double blinded studies the positive effect of PRP is less clear and results are sometimes contradictory.

1.2.3 Autologous Conditioned Sera

Many cytokines play a role in the pathogenesis of osteoarthritis, including tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (Smith *et al.*, 1989; Goldring, 2000; Hegemann *et al.*, 2005). These molecules contribute to osteoarthritis by increasing the concentration of matrix metalloproteinases that subsequently degrade the articular cartilage. Additionally, they stimulate the production of other inflammatory molecules such as nitrous oxide, cyclooxygenase-2, and prostaglandins (Alaaeddine *et al.*, 1999; Guerne *et al.*, 1999; Dahlberg *et al.*, 2000). This results in a transition of the joint to a catabolic state with degradation of cartilage matrix and chondrocyte apoptosis. The synovial membrane of dogs affected by osteoarthritis has been shown to release such cytokines including IL-1β, IL-6, IL-10, and many others (Maccoux *et al.*, 2007). This supports the hypothesis that IL-1β is involved in the pathogenesis of osteoarthritis.

As the role of IL-1β in the pathogenesis of osteoarthritis becomes clearer, the control of IL-1β protein expression and receptor binding become interesting therapeutic targets. Interleukin-1 receptor antagonist protein (IRAP or IL-1RA), is an endogenous protein produced by different organs, including joints. IRAP inhibits the activity of IL-1β by binding to the IL-1 receptor.(Hannum *et al.*, 1990; Smith *et al.*, 1991) The ratio of IL-1β to IL-1RA has been proposed to play a role in maintaining the balance between anabolic and catabolic processes within the joint, where a ratio in favour of IL-1β may promote the progression of osteoarthritis.(Carter *et al.*, 1990; Smith *et al.*, 1991; Firestein *et al.*, 1992) An injured joint likely struggles to maintain a concentration of IL-1RA that is sufficient to control the negative effects of IL-1β. Development of an IL-1RA therapeutic may therefore reduce the clinical signs associated with osteoarthritis by reducing joint damage associated with IL-1β.

Efficacy of IL-1RA in reducing progression of osteoarthritis in dogs was proven experimentally using intra-articular injection of human IL-1RA protein (Caron *et al.*, 1996) and intra-articular injection of synovial cells genetically modified to express the IL-1RA gene (Pelletier *et al.*, 1997). Unfortunately, these IL-1RA products are not available to veterinary clinicians. However, IL-1RA can also be produced by incubating (conditioning) coagulated whole blood, and incubated serum containing increased concentrations of IL-1RA is known as autologous conditioned serum (ACS) and/or IRAP. When isolating IL-1RA in this way, IL-1RA is solubilized in autologous serum which means that, similar to PRP, it is a complex and incompletely characterised blood product.

The production of ACS involves collection of 10 to 50 mL of whole blood. It is very important that the blood is collected carefully because a haemolyzed autologous conditioned sera preparation might be ineffective for clinical use. Following collection, blood is injected into a single-use vial containing a

number of borosilicate beads. The blood coagulates and the chamber is incubated at 37°C for 6 to 24 hours, depending on the system. During incubation, leukocytes are thought to adhere to the surface of the borosilicate beads, which contain a proprietary coating or etching. Leukocyte adhesion increases the expression and release of IL-1RA and other anti-inflammatory cytokines and growth factors into the coagulated blood. Following incubation, the sterile chamber is centrifuged to isolate the serum sample, which contains an increased concentration of IL-1RA compared to control (normal) serum. The ACS product is then administered as an intra-articular injection (Saunders, Bearden and Franklin, 2018).

Although most studies focus on the concentration of IL-1RA within ACS, it is important to realise that this provides an incomplete understanding of a complex biologic product. The conditioning of coagulated whole blood results in an incompletely characterized biologic product containing a myriad of growth factors and cytokines at unknown concentrations. Although some of these molecules may be beneficial to treat osteoarthritis, may also contain molecules that are harmful to the joint. Also, as for platelet-rich plasma, individual patient variation and differences in ACS systems are likely produce a variety of IL-1RA, IL-1β, and other growth factor concentrations (Huggins *et al.*, 2015; Sawyere *et al.*, 2016). For these reasons and because of the complex nature of osteoarthritis in individual joints response to treatment may vary considerably between patients.

The use of ACS as treatment for osteoarthritis in humans and horses has been reported for several years. A study from 2003 confirmed that incubation of human blood in an ACS system led to a significant increases in IL-1RA and other anti-inflammatory proteins (Meijer *et al.*, 2003). Although available literature is scarce even in human medicine, results are promising. A double-blind, placebo-controlled study involving 167 patients reported a statistically significant improvement in some of the outcome scores (KOOS and KOOS sport scores) although there were no significant differences in the major outcome measures (WOMAC score) (Yang *et al.*, 2008). In a randomized, double-blind, placebo-controlled clinical trial study comparing ACS to hyaluronic acid in 345 people with osteoarthritis of the knee, both treatment groups had a reduction in symptoms, but the ACS group showed significantly greater functional improvement up to 2 years post-treatment (Baltzer *et al.*, 2009).

In veterinary medicine, two commercially available systems (Orthokine and IRAP II) have been proven to increase IL-1RA concentration in equine serum samples (Hraha *et al.*, 2011). Orthokine was

compared to saline placebo in an experimentally induced osteoarthritis model; horses treated with weekly ACS injections showed significant clinical improvement in lameness, decreased synovial membrane hyperplasia, and an increased synovial fluid concentration of IL-1RA (Frisbie *et al.*, 2007).

Evidence supporting the efficacy of ACS in canine osteoarthritis patients is scarce. A canine ELISA was used to evaluate the effect of IRAP II system on blood samples obtained from 12 healthy dogs, and a 40- fold increase in canine IL-1RA compared to pre-treatment serum samples was identified (Huggins *et al.*, 2015). Also, there were no differences in IL-1RA concentrations when canine serum samples were stored at 4°C for 30 days or –20°C for 90 days compared to day 0. Similarly, Sawyere *et al.*, (2016) evaluated the Orthokine system using canine blood, and reported a five-fold increase in IL-1RA following incubation for 7 hours, compared to untreated serum. Importantly, this study reported that IL-1β concentrations remained similar to those of pre-treatment samples, suggesting that the conditioning process results in increased IL-1RA concentrations without undesired increases in IL-1β. Although these studies document the ability of ACS systems to isolate autologous serum with increased concentrations of IL-1RA, clinical evidence to support the use of ACS for management of canine osteoarthritis is currently limited.

In a clinical study, Hauri *et al*, (2010) describe the use of Orthokine for treatment of eleven dogs with either knee or elbow OA, and report a persistent improvement in lameness score in all patients up to three months after treatment. Results of this study needs to be interpreted with caution however, as only subjective outcome measures were adopted and there was no control group. Also, these results are contained in an abstract that, to the author knowledge, has not yet been published in any peer reviewed journal.

1.2.4 Table 1: Summary of veterinary literature

Paper reference:	System/s used:	Conclusions:	Limitations:
Thoesen, M. S. et al. (2006) 'Use of a centrifugation-based, point-of-care device for production of canine autologous bone marrow and platelet concentrates', American Journal of Veterinary Research, 67(10), pp. 1655–1661.	SmartPReP 2 system, Harvest Technologies.	This system concentrated platelets by 6-fold over baseline.	No information on clinical efficacy of the system tested.
Stief, M. et al. (2011) 'Concentration of platelets and growth factors in canine autologous conditioned plasma', Veterinary and Comparative Orthopaedics and Traumatology, 24(2), pp. 122–125.	ACP™ Double Syringe System. Arthrex Inc.	No increase in platelet concentration was noted.	No information on clinical efficacy of the system tested.
Franklin, S. P., Garner, B. C. and Cook, J. L. (2015) 'Characteristics of canine platelet-rich plasma prepared with five commercially available systems', American Journal of Veterinary Research, 76(9), pp. 822–827.	1. Protec PRP, PulseVet; 2. MediVet PRP, medivet America; 3. V-PET, Pall Corporation. 4. SmartPReP2, harvest technologies; 5. Angel, Arthrex Vet Systems,	All but one system (System 1) produced a product with an increased platelet concentration.	No information on clinical efficacy of the systems tested.
Carr, B. J. et al. (2016) 'Canine Platelet-Rich Plasma Systems: A Prospective Analysis', Frontiers in Veterinary Science, 2(January), pp. 1–8.	1. SmartPReP2, Harvest Technologies; 2. Arthrex ACP, Arthrex; 3. CRT Pure PRP, Canine Regenerative Therapies; 4. ProTec PRP, Pulse Veterinary Technologies; 5. V-PET, Pall Corporation.	Significant increase in platelet concentration over baseline was noted only for systems number 1 and 5.	No information on clinical efficacy of the systems tested.
Frye, C. W. et al. (2016) 'Assessment of canine autologous platelet-rich plasma produced with a commercial centrifugation and platelet recovery kit', Veterinary and Comparative Orthopaedics and Traumatology, 29(1), pp. 14–19	Terumo APC-30 processing kit (APC-30): Terumo Medical Corporation.	This system concentrated platelets by 6-fold over baseline	No information on clinical efficacy of the system tested.
Silva, R. F., Carmona, J. U. and Rezende, C. M. F. (2013) 'Intra-articular injections of autologous platelet concentrates in dogs with surgical reparation of cranial cruciate ligament rupture', *Veterinary and Comparative Orthopaedics and Traumatology*, 26(4), pp. 285–290.	PRP produced by centrifugation without use of a point of care kit.	Ten dogs with cranial cruciate ligament (CCL) rupture that underwent arthroscopic replacement of the ligament with fascia lata, were treated with either three intra-articular injections of PRP two weeks apart or with an oral nutraceutical. Gait analysis, clinical examination and	Low patient number

		radiographs were repeated at monthly intervals for 3 months. No difference was noted in the radiographic scores, but at 3 months dogs that received PRP had higher clinical scores and higher force plate metrics.	
Franklin, S. P. and Cook, J. L. (2013) 'Prospective trial of autologous conditioned plasma versus hyaluronan plus corticosteroid for elbow osteoarthritis in dogs', Canadian Veterinary Journal, 54(9), pp. 881–884.	ACP Arthrex	Ten dogs with bilateral elbow osteoarthritis were randomly divided in two groups and treated with either one intra-articular injection of PRP or an intra-articular injection of a combination of hyaluronic acid and steroids. Outcomes were evaluated via validated questionnaires and subjective blinded lameness assessment. Dogs in both groups improved over time and although some owner questionnaire had higher score in the PRP group, no statistical difference was noted between the two groups.	Low patient number Lack of control group No objective outcome assessment
Fahie, M. A. et al. (2013) 'A randomized controlled trial of the efficacy of autologous platelet therapy for the treatment of osteoarthritis in dogs', J Am Vet Med Assoc, 243(9), pp. 1291–1297.	V-PET, Pall Corporation.	Twenty dogs with clinical signs of osteoarthritis in a single joint were treated with one intra-articular injection of either PRP or saline.. All patients were evaluated via owner assigned lameness scores and pain scores. Three months after treatment no improvement was noted in any of the parameters for the control group while a significant improvement was noted in all the parameters for the PRP group	Force plate analysis performed only in one half of patients. Low patient number
Venator, K. et al. (2020) 'Assessment of a Single Intra-Articular Stifle Injection of Pure Platelet Rich Plasma on Symmetry Indices in Dogs with Unilateral or Bilateral Stifle Osteoarthritis from Long-Term Medically Managed Cranial Cruciate Ligament Disease', Veterinary Medicine: Research and Reports, 11, pp. 31–38.	Terumo APC-30 processing kit (APC-30): Terumo Medical Corporation.	Ten dogs with chronic CCL rupture were treated without surgery with a single injection of PRP and their progress monitored by mean of a pressure sensitive walkway. The authors reported improvement of symmetry index but no significant change of peak vertical forces (PVF) or vertical impulse	Low patient number, Lack of control group
Ho, L. K. et al. (2015) 'Single ultrasound-guided platelet-rich plasma	Harvest SmartPReP, Harvest Technologies	A case series with 10 dogs reported the use of	Low patient number

injection for treatment of supraspinatus tendinopathy in dogs', The Canadian veterinary journal = La revue veterinaire canadienne, 56(8), pp. 845–849		ultrasound guided injection of PRP for the treatment of supraspinatus tendinopathy. Although 6 weeks after treatment ultrasonographic features of the tendons improved in 6 patients, and 4 patients had subjective improvement of lameness reported by the owner, there was no objective improvement based on kinetic gait assessment.	Lack of control group No objective outcome assessment
Hraha, T. H. et al. (2011) 'Autologous conditioned serum: The comparative cytokine profiles of two commercial methods (IRAP and IRAP II) using equine blood', Equine Veterinary Journal, 43(5), pp. 516–521.	IRAP, Arthrex. IRAP II, Arthrex.	The cytokine profile that IRAP II produced is modestly better than IRAP. Incubation of whole blood in glass tubes stimulated cytokine synthesis, although not as efficiently as IRAP II.	No information on clinical efficacy of the systems tested.
Huggins, S. S. et al. (2015) 'Serum concentrations of canine interleukin-1 receptor antagonist protein in healthy dogs after incubation using an autologous serum processing system', Research in Veterinary Science. Elsevier, 101, pp. 28–33.	IRAP II, Arthrex.	A canine ELISA was used to evaluate the effect of IRAP II system on blood samples obtained from 12 healthy dogs. This resulted in a 40 fold increase in canine IL-1RA compared to pre-treatment serum samples	No information on clinical efficacy of the system tested.
Sawyere, D. M. et al. (2016) 'Cytokine and Growth Factor Concentrations in Canine Autologous Conditioned Serum', Veterinary Surgery. Blackwell Publishing Inc., 45(5), pp. 582–586.	Orthokine, Orthogen	Incubation for 7 hours resulted in a fivefold increase in IL-1RA compared to untreated serum	No information on clinical efficacy of the system tested.
Hauri, S. (2010) 'Autologous conditioned serum generated with the irap device. a new therapy for dogs', Wsava, 68(3), pp. 1–3.	Orthokine, Orthogen.	Treatment of eleven dogs with either knee or elbow OA and report a persistent improvement in lameness score, in all patients up to three months after treatment.	Only subjective outcome measures adopted Lack of control group. Results not published on peer review journal

1.3 ASSESSMENT OF CLINICAL JOINT DISEASE

1.3.1 Gait Analysis Systems

1.3.1.1 Overview

The gait cycle consists of two main phases: the stance phase, when the paw is in contact with the ground, and the swing phase, when the foot is in the air. During the stance phase the paw exerts a force on the ground and the ground reacts with an equal and opposite force (Newton's third law), the *ground reaction force,* which comprises all forces acting on the paw of the animal (measured in Newtons). This is not specific for any one joint and can be described by a three-dimensional force vector: vertical (F_z), craniocaudal (F_y), and mediolateral (F_x) (DeCamp, 1997). Studies often focus on the vertical force (largest and closely associated with bodyweight) and more rarely on the craniocaudal

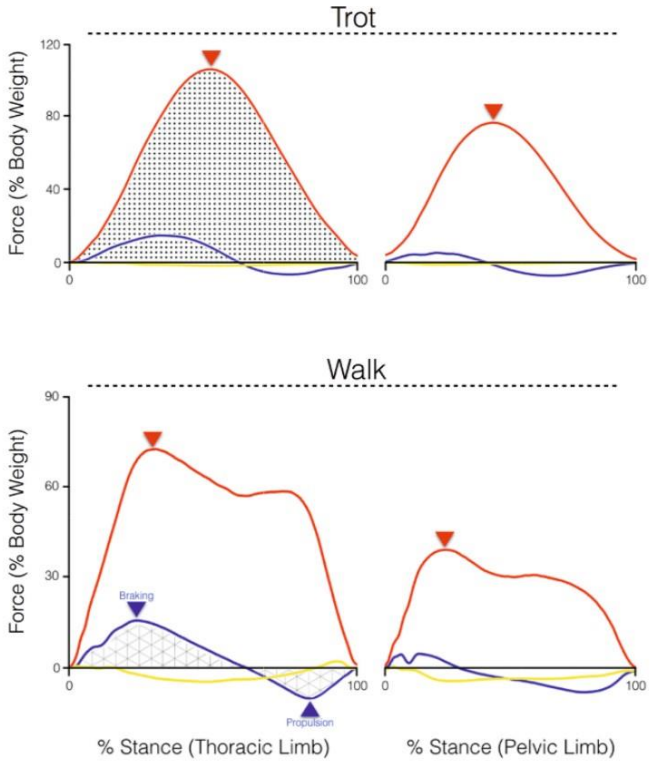


Figure 1 Graphic representation of ground reaction forces at the trot and at the walk. The red line represents the vertical force (Fz), the blue line represents the craniocaudal force (Fy), and the yellow line represents the mediolateral force (Fx). The peak force is labelled with a red arrowhead for the vertical (Fz) force during the walk and trot. The craniocaudal peak forces (Fy) are labelled with a blue arrowhead for the walk. The vertical impulse is depicted for the thoracic limb trot graph with a dotted area under the curve. The craniocaudal impulses are labelled for the walk with a cross-hatched area under the curve. **Adapted from *"Veterinary Surgery, Small Animal, second edition"* by S.A. Johnston and K.M. Tobias, 2018, page 1386. Copyright 2018 by Elsevier.**

force (braking and propulsive force). The mediolateral force is rarely studied as it is small and inconsistent (Rumph *et al.*, 1995).

Canine gait is made of a series of coordinated movements and gaits are typically divided into symmetrical gaits and asymmetrical gait (DeCamp, 1997). In symmetrical gaits (walk, trot, and pace) the limb movement on one side is repeated identically on the opposite side and for this reason gait analysis focuses mostly on symmetrical gaits. In asymmetrical gaits (canter, transverse gallop, and rotary gallop) limb movement on one side is not exactly repeated on the opposite side.
Asymmetrical gaits are rarely analysed because they are less relevant for dogs and also, because of their asymmetry, much more complex to study.

Gait analysis in veterinary patients presents additional challenges compared to human medicine. Veterinary surgeons treat a variety of species which are morphologically very different one from the other. Also, even within the same species such as the dog, morphology can vary significantly from one patient to the other. Despite these challenges, objective gait analysis techniques have become increasingly used in veterinary medicine over the last few decades.

The two fields of gait analysis are kinetics and kinematics. Kinetic gait analysis studies the forces generated by the limb against the ground, while kinematic gait analysis studies three-dimensional motion of body and trunk in relation to space and time, irrespective of the forces involved. Most studies use kinetic gait analysis as robust data is simpler to collect – the animal has only to be walked across a plate, for example, compared to kinetic systems where markers have to be firmly attached to various anatomical landmarks on the animal, which must remain in view of multiple cameras.

Kinetic data are often displayed graphically for easier interpretation: the vertical force in dogs produces a “bell shape” curve during the trot and a “M shape” curve during the walk. This difference in shape is due to the higher speed at which events occurs and are recorded at the trot compared to the slower walk (DeCamp, 1997). The second largest force is the craniocaudal force (F_y). This defines the braking (deceleration) and propulsion (acceleration) of the walks which happen respectively at the beginning and end of the stance phase – during the cross-over point, the force is zero. The mediolateral force (F_x) is small and variable and so rarely considered/reported in gait studies (DeCamp, 1997).
The most commonly reported values in gait analysis are the peak force and impulse values. Peak force is the highest force exerted in a particular direction (i.e. vertical, craniocaudal and mediolateral), while the impulse is the area under the force-time curve, and therefore dependant on both force and

contact time. These values are both accepted as measures of limb function and pain (McLaughlin, 2001; Souza *et al.*, 2015). Lameness or limb pain cause a reduction in weight bearing and also a reduction of the time that weight is applied to the leg therefore lowering the values of both peak vertical force and vertical impulse. In contrast to the vertical force, which is described in a single phase, the craniocaudal force (F_Y) consists of both a breaking and a propulsive phase. Both braking and propulsive phases are shortened in dogs with cranial cruciate ligament insufficiency (Rumph *et al.*, 1995). A similar shortening is noted in animals with thoracic limb lameness, where the reduction in braking phase is more marked than that of the propulsion phase (Figure 1) (Abdelhadi *et al.*, 2012).

Other parts of the force curves that can provide information on limb function, but are studied less frequently, are the rising and falling slope which represent the loading and offloading phases of weight application to the ground. The rising slope describes the period from initial paw contact to the peak force. A steeper slope indicates a faster loading of weight on the limb, while a less steep slope indicates a slower loading of weight onto the limb. The falling slope represents the period from the peak force to the point at which the paw is lifted and ground contact stops, and represents the unloading of weight from the limb.

Kinetic gait analysis data have proven useful in describing and studying both healthy dogs and also dogs with orthopaedic disease. In healthy dogs, such data have facilitated understanding of how weight is distributed across the limbs during stance, and different gaits (Souza *et al.*, 2015). In dogs affected by orthopaedic disease, kinetic gait analysis has enabled evaluation of the effects of different management options such as drug therapies (Vasseur *et al.*, 1995), diet (Mlacnik *et al.*, 2006) and weight loss (Marshall *et al.*, 2010).

Measurements of PVF and VI are rapidly obtained, easily compared, and provide valuable information that is clinically useful. It is important to bear in mind that measurement of PVF and VI ignore the other ground reaction forces, and subtle changes that occur throughout the stance phase may be overlooked (Al-Nadaf, Torres and Budsberg, 2012).

Kinetic data are routinely normalised to body weight. This process reduces the variability of kinetic data due to patient weight differences within a study population but does not completely eliminate it (Voss *et al.*, 2010). In normal standing dogs, forelimbs support approximately 60% of the body weight while hindlimbs support approximately 40% (Voss *et al.*, 2011).

Dynamic gait data can be obtained at the walk and trot. Both are symmetrical gaits and can be used to differentiate lame from non-lame dogs. However, the trot has been shown to be more sensitive and accurate for the detection of lameness in dogs with low-grade or mild lameness. Additionally, during the trot there is no overlap of footfalls on the force plate / pressure mat making easier to evaluate each limb. The advantages of each gait for kinematic analysis is a matter of ongoing debate (Voss *et al.*, 2007).

It is important that patient speed and acceleration are controlled to limit variability of the gait, as peak forces, for example, tend to be higher at faster gaits. However, there is no consensus regarding the ideal velocity for a trot or walk in dogs. In one study, different trotting velocities ranging from ±0.3 m/s to ±1.0 m/s, with an average of ±0.6 m/s were reported (Hans *et al.*, 2014).

There is limited information on the effect of acceleration on kinetic data. Changes to acceleration/deceleration have been shown to alter ground reaction force measurements and this is most apparent in the craniocaudal force values. Acceleration is therefore typically controlled at ±0.5 m/s^2 in kinetic gait studies (Budsberg, Rytz and Johnston, 1999).

Ground reaction forces can be interpreted by comparing data obtained from one limb to the contralateral limb, generating a "Symmetry index". Symmetry indices involve using the patient's contralateral limb as an internal control for comparison. In normal dogs, gait is assumed to be symmetrical, and a lack of symmetry is assumed to be associated with pathologic gait. However, a degree of asymmetry may be present even in normal dogs which is likely to be caused by trial-to-trial variation rather than true variation between contralateral limbs (Budsberg *et al.*, 1993). Because of this, normal levels of asymmetry have been suggested to be <3.2% (Fanchon and Grandjean, 2007), or <6% (Clough *et al.*, 2018). Equally, symmetry indices need to be interpreted with caution when bilateral orthopaedic disease is present, as a symmetric gait in these patients might indicate bilateral lameness, rather than absence of lameness.

There are many sources of variability in kinetic gait analysis that have been studied. These include: variance attributed to the patient (Jevens *et al.*, 1993), handler (Jevens *et al.*, 1993), trial repetition (Jevens *et al.*, 1993), habituation to the testing area (Rumph, Steiss and Montgomery, 1997), and extreme changes in velocity (McLaughlin and Roush, 1995), stance time, or acceleration/deceleration (Budsberg, Rytz and Johnston, 1999).

Most kinetic gait analysis studies are performed using a "force plate" which is currently considered the gold standard.

1.3.1.2 Force plates

A force plate uses transducers to measure the forces that applied against it. There are different types of transducers; most force plates use either strain gauge or piezoelectric sensor transducers. It is possible to use a single force plate or multiple in-line force plates. A single force plate can usually only acquire data from one footfall at the time. For this reason multiple in-line force plates enables data to be collected from on a greater number of footfalls with a single pass, reducing overall collection time, trial repetition, and also variability due to patient fatigue.

If more than one paw contacts the force plate at the same time, the force traces will overlap, making interpretation more difficult. The peak forces are still identifiable as separate and distinct points; however, the cranio-caudal forces and impulses are less useful due to the overlap. Standard-sized force plates work well for medium- and large-breed dogs while small dogs and cats are likely to strike the force plate simultaneously with more than one limb. To prevent this, the use of a custom built platform that reduces the exposed area of the force plate has been described (Kapatkin *et al.*, 2014).

Patient velocity and acceleration can be measured with the use of photocells which emit an invisible photoelectric beam. On the other side of the gait platform, opposite to each photocell is a reflector that reflects the beam. The time when each beam is interrupted by the animal allows calculation of both average velocity and acceleration across the known distance between them. A minimum of two photocells are required to measure patient velocity while three photocells are required to measure acceleration. The majority of force plate systems in veterinary practices uses three to five photocells (Punke *et al.*, 2007).

1.3.1.3 Pressure walkway systems

Although force plates are considered the gold standard in research gait laboratories, pressure walkways are increasingly used in clinical gait analysis. A major strength of pressure walkways is their portability, and the fact that they can be placed on top of a floor, without the need to be *set into* the floor. Some systems can be rolled up or disassembled for storage and transportation. Pressure walkways are available in various lengths, and there are modular systems that can be lengthened or shortened by adding or removing sections to meet the needs of the clinician. Compared to force plates, the overall structure of pressure walkways allows easier evaluation of animals of different sizes and body morphology. Evaluation of small animals with shorter stride lengths can be challenging using

a force plate and physical alteration to the gait platform might be required to isolate overlapping footfalls on a single plate (Kapatkin *et al.*, 2014). On the contrary, pressure walkways can record simultaneous information from each individual footfall. Pressure walkways also allow collection of multiple gait cycles in one pass, leading to faster data acquisition (Lee *et al.*, 2002) and, potentially, less subject fatigue.

The main difference between pressure walkways and the more traditional force plate is how force data are acquired. Pressure walkways contain several hundred pressure sensors which can record in real time the pressure across all areas of the walkway. This means that when two or more limbs strike the walkway at the same time, the system can acquire and interpret data from each limb individually; this is not possible with force plates. Pressure, however, can be recorded only in the vertical direction and pressure walkways cannot be therefore be used to measure force in craniocaudal and mediolateral directions. Pressure is reported in Pascals in contrast to force which is reported in Newtons, but some walkway systems do allow the conversion of pressure values to vertical forces, following a calibration process.

Vertical force values obtained from force plates and a pressure walkway are not directly comparable. However, walkways produce consistent and repeatable measurements enabling evaluation of patients over time (Lascelles *et al.*, 2006). Pressure walkways have been used to study dogs with cranial cruciate ligament disease (Horstman *et al.*, 2004); hip dysplasia (Upchurch *et al.*, 2016); and hip replacement surgery (Lascelles *et al.*, 2010); and for gait analysis in cats (Verdugo *et al.*, 2013; Schnabl and Bockstahler, 2015), and pigs (Meijer *et al.*, 2014).

In contrast to well-established force plate systems, studies are lacking on sources of variability in data collected using pressure walkway systems. Additionally, standard collection techniques and protocols need to be defined to enable comparison of pressure walkway data from patients at different hospital locations. A significant part of the research behind this masters project was the development of standard protocol for calibration and use of the pressure mat. It was concluded that, using the proposed protocol, results were highly reproducible and repeatable and not affected by different operators (Rincon Alvarez *et al.*, 2020). While these results support the use of a pressure walkway in clinical settings, the study did not investigate sources of variability of pressure walkway data, or repeatability of data acquired by different walkways in different hospitals. These remains sources of possible variability that need to be investigated.

Despite pressure walkway limitations in assessing force, they enable collection of temporospatial parameters such as stride time, stance time, walking velocity, and the calculation of symmetry indices, which are challenging to calculate accurately with force plate systems.

There is limited information in the current literature on the optimal environment for collection of gait data using a PSW. It has been recommended that a designated room is identified, with 3 to 4 metres on either side of the walkway (Romans *et al.*, 2004; Lascelles *et al.*, 2006; Lascelles *et al.*, 2007; Kim, Kazmierczak and Breur, 2011). As a general rule, it is accepted that PSWs should be located in a quiet space, with enough space on either side of the PSW allowing the dog to access it at a constant velocity and to leave it without stopping abruptly.

1.3.2 Electronic von Frey Anaesthesiometer

To investigate more thoroughly the possible effects of the treatments under investigation in this Masters project, an electronic Von Frey anaesthesiometer (VFA) was used to try identify any change in chronic central pain sensitization.

Somatosensory abnormalities can be assessed using quantitative sensory tests (QSTs), which involve the application of mechanical, thermal or electrical stimuli to an area to assess sensory and/or pain pathways (Tomas *et al.*, 2014). A VFA is a device used for quantitative sensory testing (QST) that tests sensory threshold for a punctate mechanical stimulus, in this case, a plastic pressure probe applied to the skin. The device consists of a hand-held applicator with a plastic tip, a load cell, and a recording device. Mechanical force is applied to a surface via the handpiece and a plastic tip and is registered by the load cell which transmits a measurement of load (measured in grams) to an electronic recording device.

In chronic painful states such as osteoarthritis, central sensitisation has been identified due to sustained activity of nociceptors, leading to an increase in the excitability of neurons within the central nervous system. This activity-dependent synaptic plasticity leads to increases in synapse efficacy and reductions in inhibition, causing somatosensory abnormalities such as allodynia, hyperalgesia or thermal hypersensitivity both locally, and at sites remote to the affected joint (Woolf, 2011). Central sensitisation is a potentially important component of the pain response, which has implications for the diagnosis and effective treatment of pain. Central sensitization (CS) as a result of OA is recognized as an important facet of chronic pain in human patients and has been measured in people using quantitative sensory testing (QST) testing (Knazovicky *et al.*, 2016).

In veterinary settings a Von Frey anaesthesiometer was used successfully to identify the presence of central sensitization in dogs in association with cruciate ligament rupture(Brydges *et al.*, 2012), hind limb OA (Williams *et al.*, 2014; Knazovicky *et al.*, 2016) and neuropathic pain (Kerns *et al.*, 2019) and also in cats with hindlimb OA (Addison and Clements, 2017).

To the authors knowledge, the presence of central sensitization secondary to elbow osteoarthritis in dogs have not been evaluated. The author has started a collateral project, which is currently underway, to specifically investigate this further.

1.3.3 LOAD questionnaire

A further component of this Master's project was to assess the effect of the treatment as perceived by the patients' owners. Owner's assessment of lameness/pain and limb function is subjective, and it is well recognised that using owners to assess the effects of an intervention (regardless of the type of treatment) is complicated by several factors that can introduce bias (Glaser *et al.*, 1997; Bowling, 2005; Choi and Pak, 2005; Cook, 2010). However, the importance of client satisfaction with the outcome of any veterinary treatment should not be underestimated. To try to address this subjectivity to a degree, owner assessments are often undertaken using a clinical metrology instrument (CMI), also called a questionnaire. Using a validated questionnaire is essential in decreasing the effect of the biases (Marx *et al.*, 2003).

There are at least six CMIs reported for measuring the severity of OA in dogs (Innes and Barr, 1998; Brown et al., 2008; Hercock et al., 2009a; Hielm-Björkman and Rita, 2009). The authors elected to use the The Liverpool Osteoarthritis in Dogs (LOAD) for this project as it is It is convenient to use, validated and the results are also correlated with force-platform data (Walton et al., 2013). LOAD was originally tested on dogs with elbow osteoarthritis (Hercock *et al.*, 2009b) and more recently it has been extensively validated in a study involving over two hundred owners of dogs with arthritis of the elbow, hip, and stifle (Walton *et al.*, 2013).

The LOAD questionnaire is a clinical metrology instrument (CMI) composed of 13 questions.(Muller *et al.*, 2016a) Individual question scores are summed and produce a "LOAD score" which is suggestive of the animal's disease severity.

The above references highlight that the main strengths of the LOAD questionnaire are:

- it can be recommended for the assessment of canine osteoarthritis
- it is convenient to use
- it has been validated by peer review
- it can be correlated with force-platform data

The LOAD questionnaire is attached at the end of this Master thesis (Appendix 5).

1.4 AIM AND HYPOTHESIS

The main aim of this research project was to improve the currently limited evidence on the effectiveness of V-PET and Orthokine, for the treatment of elbow osteoarthritis in dogs by comparing the pre and post treatment values of pressure mat data, VonFrey anesthesiometer data and LOAD questionnaire data.

Our hypothesis was that both treatment options would have led to statistically significant improvement of all outcome measures when posttreatment values were compared to pretreatment values.

2 MATERIALS AND METHODS

2.1 ETHICS APPROVAL AND ANIMAL TEST CERTIFICATE

Prior commencing data collection approval of the ethical committee of the university of Glasgow was granted on the 6th November 2018 with reference number 40a/18 (Appendix 6).

Although the products used in this project are commercially available for treatment of canine elbow disease, the ethical committee raised the concern that an Animal Test Certificate from the Veterinary Medicine Directorate might be legally required.

The author contacted the Veterinary Medicine Directorate asking for clarification and was advised that an Animal Test Certificate for small scale non-commercial trials (ATC-S) should be obtained before commencing the trial. This was requested and granted on 26th February 2019 with reference number ATC-S-114 (Appendix 7). Due to the delay of covid19 pandemic on data collection, the author applied for a two years extension of the ATC which was granted on the 26th February 2021.

2.2 INCLUSION CRITERIA

- Dogs with a history of forelimb lameness due to elbow pain of at least six months' duration and radiographic or CT evidence of elbow osteoarthritis. Dogs with both unilateral and bilateral disease were accepted, as long as cases with bilateral disease had one more severely affected leg.
- Absence of any other symptomatic musculoskeletal disorder. Those patients with other orthopaedic conditions were included if, based on orthopaedic exam, the elbow was considered to be the main cause of the lameness.
- Medium, large breeds only (20-45KG), with body condition score between 4/9 and 6/9. Overweight and obese patients could be included only if owner declared that diet plans had already been attempted and failed, as weight management should always be the first line of treatment.
- Patients up to 12 years of age.
- Persistent lameness despite medical management - treatment must not have changed in the two months before inclusion, as the dog will act as its own control. Patients on NSAIDS, or other medications, will continue with the medication unaltered for the duration of the study.
- No orthopaedic surgery (including elbow arthroscopy) performed in the previous three months
- Patient deemed to be healthy on clinical examination, with no contra-indications to receiving medetomidine sedation or intra-articular injection. (e.g. patients with a heart murmur or skin conditions would be excluded). Patients should have had haematology and biochemistry done within last 3 months – if not, a pre-anaesthetic panel will be performed in-house.

2.3 PRESSURE SENSITIVE WALKWAY

2.3.1 Disclosure

This section on the pressure sensitive walkway (PSW) is reproduced with permission and minor modifications from the MVM Masters thesis of Javier Rincon Alvarez (JAR)(Rincon Alvarez, 2021). Both authors (JAR and Simone Anesi) contributed equally to setting up and validating the pressure walkway, and optimising a protocol for its use, prior to each student undertaking separate studies (JAR on repeatability of pressure walkway data, SA using it to collect objective data on outcomes following elbow OA therapies).

2.3.2 Specifications and components

The PSW consists of a low profile, high-definition system of three sequentially connected plates, with embedded pressure sensors called “sensels” (Strideway HRSW3, Tekscan, South Boston, USA). The sensels produce a raw digital output when they are stimulated by the animal’s weight; the digital output is subsequently converted by specific software (Strideway Research, Tekscan, South Boston,

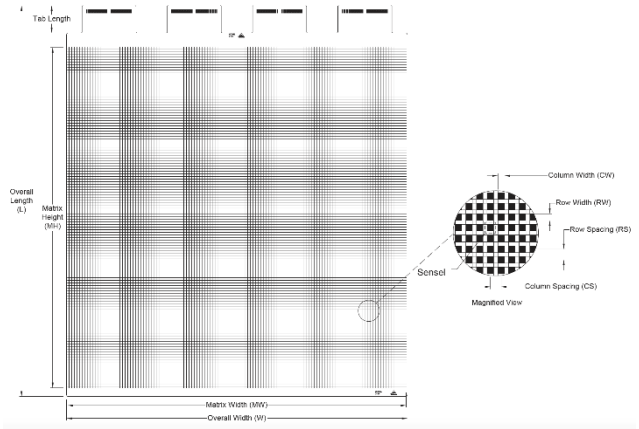


Figure 2 Representation of sensel disposition on the pressure plate. Image from Tekscan Strideway User Manual

USA) into pressure units. On each plate, the sensels are arranged in columns and rows separated from each other by 1.9 mm of “empty” space, creating a honeycomb-like dense panel of sensels. The separation between sensels and the honeycomb-like distribution determines how the PSW will interpret the applied load, and therefore the final pressure output. When a load is applied to the plate’s surface by a material that can undergo deformation e.g. a bare foot or foam, part of the load will “sink” into the empty space between sensels. On the other hand, if a material that does not undergo deformation is applied e.g. shoes, a stool or hard plastic, the entire load will lie over the

sensels, with none “sinking” in the spaces within them. This same load will be interpreted differently by the software.

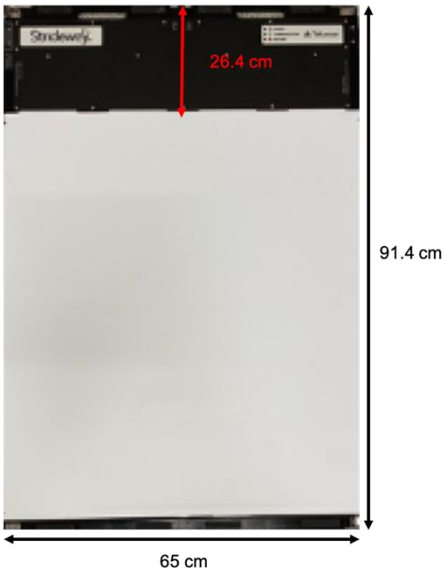


Figure 3 Tekscan plate. Note the grey area containing the sensels and the black area containing the hardware. Image from Tekscan Strideway User Manual

Each plate measures a total 65.0x91.4x1.5 cm, with an area of 65.0x26.4x1.5 cm containing the hardware i.e. USB connector, power input connectors and microchips (Part in black in Figure 3). Therefore, the active sensel surface of each plate was 65.0x65.0x1.5 cm, with a sensel density of 3.88 sensels/cm2 (Part in grey in Figure 3). Three sensing plates were linked together, and then a tapered non-pressure sensitive plate measuring 65.0x91.4x1.40 cm was added to each end to create a smooth transition from the ground to the walkway. Therefore, the runway length was 325 cm (i.e., 65cm x 5 plates in total) with a pressure sensing length of 195 cm (i.e., 65cm x 3 sensing plates), containing 48768 sensels.

The entire walkway was covered by a 0.3 mm thick rubber matt to protect the plates and prevent the dog from slipping. This mat was specifically designed and supplied by the manufacturer, and secured by “Velcro” attachments located all along the plates.

The PSW was connected to a dedicated computer (Lenovo 81 AX, Quarry Bay, Hong Kong) containing a specific Strideway Research software. A high-definition, wide angle video camera (LifeCam Cinema, Microsoft, Washington, USA) was also connected to the computer, and synchronized with the PSW by the Strideway Research software. The camera was positioned halfway along the PSW’s length,

approximately 97.5 cm from the first active sensing plate and 60 cm away from the edge of the walkway to capture the length of the walkway (Figure 4).

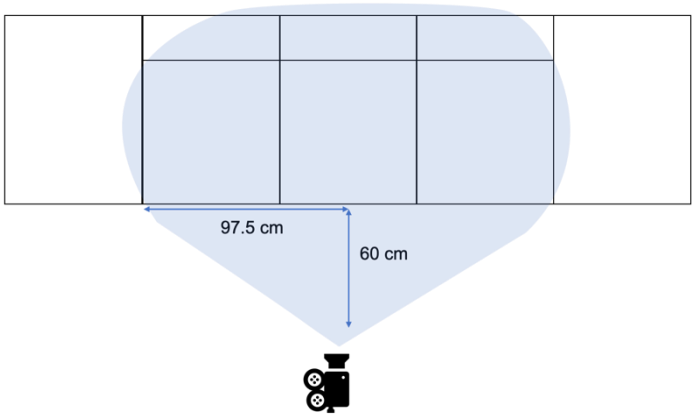


Figure 4 Representation of the PSW setting. Blue: area captured by wide-angle video camera

2.3.3 Sensitivity

Three sensitivity settings are available for the PSW: low, medium and high. The sensitivity settings determine the level of response (raw digital output) the sensels generate in response to a given load. For example: with a sensitivity setting of '1' 1 bit equals 1 mmHg with a range of 0 to 255 mmHg. When the sensitivity is adjusted to '2', 1 bit equals 0.5 mmHg resulting in a finer resolution but narrower range (0-127 mmHg). However, when sensitivity is adjusted to '0.5', 1 bit equals 2 mmHg resulting in a coarser resolution but broader range (0-510 mmHg). This allows the sensels to avoid failing to register, or, conversely becoming saturated, accordingly the subject of study. In studies with animals, it is important to adjust the sensitivity to the most appropriate setting for the evaluated animal, based on the animal's bodyweight. This process is best explained with the following example.

A 26 kg dog is walked across the PSW with the sensitivity pre-set at "low". The raw digital output interpreted by the software shows several oversaturated sensels (Figure 5a). These sensels will not be taken into account in the calculation of the pressure, producing an inaccurate result (under-estimating the pressure). This sensitivity is therefore too high for this given animal, an although the definition is very good the range of raw digital output is too narrow. When the sensitivity setting is adjusted to "high", the same dog produces a digital output that only reaches the lower aspect of the raw digital

output range (Figure 5 c). The sensitivity is therefore too low for this animal, and although the range of raw digital output is greater, the definition is too low. This will also produce an inaccurate result.

Once the sensitivity setting is adjusted to “medium”, the same dog produces a digital output that covers most of the raw digital output range, with no oversaturated sensels (Figure 5 b). Therefore, “medium” sensitivity setting should be selected for this specific dog based on the raw digital output produced on each setting.

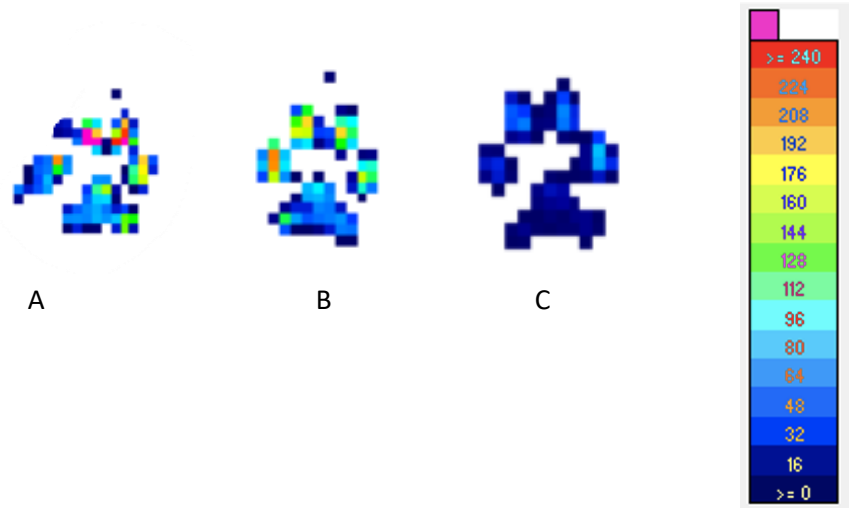


Figure 5 Three paw prints of the same dog on three different sensitivity settings. Red and pink represents oversaturation A: low; B: medium; C: high

The manufacturer recommended sensitivity settings of low, medium or high were used for small, medium and large animals respectively, however, none of the dogs in this study produced a digital output high or low enough to use the “high” or “low” settings, therefore “medium” setting was used for all cases. Despite this, the author recommends that sensitivity is selected on an individual basis for each dog prior to collection of any data, following the above protocol.

2.3.4 Calibration

Calibration is the method by which the software ‘acquires’ the information necessary to convert the raw digital output of the sensels into specific pressure units (i.e., KPa, PSI or mmHg).

Three different calibration methods can be used for the Tekscan walkway, all involving the application of a known weight for different times, with each plate being calibrated individually. These are described as follows:

1. Point calibration

A subject of know weight is used to calibrate the system. The subject stands on the plate for at least one second during which time the software generates a curve showing visually the raw output of the sensels over time. After this the operator selects a point on the curve and associates it with the known weight. To use this method, the study subject must be used to calibrate the plate, and the walkway should be calibrated again for every subject.

Counterintuitively, when a weight is applied on the walkway and left immobile for several seconds the raw output does not remain stable but decreases progressively over time (this phenomenon is defined "output drift") due to "fatiguing" of the sensels. The manufacturer and the authors do not recommend using point calibration method for gait analysis as it does not take output drift into account.

2. Frame calibration

This method can be performed only after the data have been recorded. The operator identifies a frame within the recorded data that represents the body weight of the study subject and manually associates it to the subject weight into the programme software. For quadrupedal gait analysis, it is somewhat complicated to select a single frame which will represent the body weight of the patient. This is due to the fact that several limbs are placed on the PSW at the same time. This method may be useful when force plate data are available simultaneously, as the force (weight) given by the force plate at a specific instant can be related accurately to the walkway data. However, this method is less useful in clinical setting and similarly to "point calibration" method does not account for output drift.

3. Step calibration

The known weight used for this calibration technique can be the patient/animal, an operator, or an inanimate object e.g., weighted disc. The known weight is applied to each plate of the walkway for 10 seconds. During this time the software associates the raw data output with the known weight (input into the system by the operator), accounting for the output drift. For most types of research, this is considered the most accurate technique. Additionally, as the known weight does *not* require to be the subject of study, the calibration file can be applied to the data after it has been acquired, providing calibration and data collection were both undertaken with the PSW set at the same sensitivity level.

Step calibration is considered to produce the most accurate results, as this method accounts for the "output drift" compensating automatically for the change in sensel output over time.

The manufacturer recommends the use of step calibration in animal studies. However, using the patient to perform the step calibration can be challenging, as it requires the animal to step on to the plate and remain stable for 10 seconds, which in the majority of cases is not possible. Two alternatives are commonly used to provide a known weight:

1. Human: the operator stands on the plate during the calibration process, but must balance throughout the process, and so stabilisation with a nearby vertical object (e.g. a wall or cane) has been proposed (Figure 6 a).

2. Phantom: a short three-legged device, consisting of an equilateral wooden triangle with the three short legs each with a soft 23.6 cm^2 base. This device provides better stability as the short legs are equidistant from the centre of the device. Either a willing assistant or known weight-discs can be applied to the device when performing the step calibration (Figure 6 b).

Figure 6

a. Representation of step calibration performed by an operator.

b. Representation of step calibration performed by an operator using a phantom.

2.3.5 Pressure sensitive walkway hardware set up

The first sensing plate (far left) is placed on a flat surface (floor) and lined up with one of the non-sensitive end plates (Figure 7). The plates are then attached to each other via two metallic latches.

Figure 7 First pressure sensitive plate aligned with the non-pressure sensitive end plate (to left)

The next plate is positioned to the right of the first plate. Both plates are lined up and linked by gently pulling on the connector of the plate positioned on the right. This connector will stretch to approximately 6.3 cm, and clip onto a docking site on the plate on the left (Figure 8). Care is taken not to overstretch the connector which could result on malfunction of the walkway.

Once connected, the right plate is slid to the left so both plates are flush with each other, and attached together with the metallic laches (Figure 9).

Figure 8 Detail of the connection between pressure plates

Figure 9 Detail of pressure plates connected and secured with the metallic latch

This process is repeated with the third sensing / active plate; the second non-sensitive end plate is then connected to the right end of the walkway (Figure 10).

Figure 10 PSW formed by three pressure sensitive plates and two (non-pressure sensitive) end plates

When the three plates are in place and connected to each other, the power source is connected to the left plate. a red led light indicates correct power connection. After 10 seconds, the USB cable is then connected from the computer also to the left plate. A green led light confirms correct data connection and the Strideway software programme is initiated.

Lastly, the plates are covered with the rollout rubber protective cover (Figure 1112). Particular care is taken not to form any ripples, as this could affect the data collection.

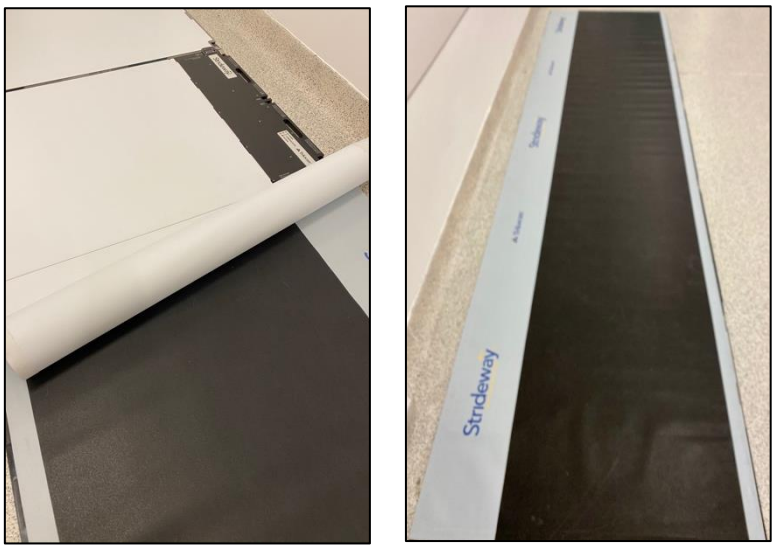


Figure 1112 PSW covered with the protective rubber cover.

Figure 1211 Final set up of the PSW. Note the tripod and camera on the floor on the right side of the corridor.

The PSW used for this Masters project was the latest version of the Tekscan® system, which is a high-definition system with the highest available sensel density and total sensel number. The system is portable, but when in use, it was set up in a quiet corridor in the Small Animal Hospital, more than 5 metres long and with at least 2 metres of space on each side of the walkway. (Figure 1211)

2.3.6 Pressure sensitive walkway software set-up

1. Patient registration

After connecting the pressure mat to the dedicated laptop, the proprietary software “Strideway Research” is launched. A new patient file is generated by clicking on File, New patient. (Figure 13)

Patient information is now input into the Patient Record-New Patient window.

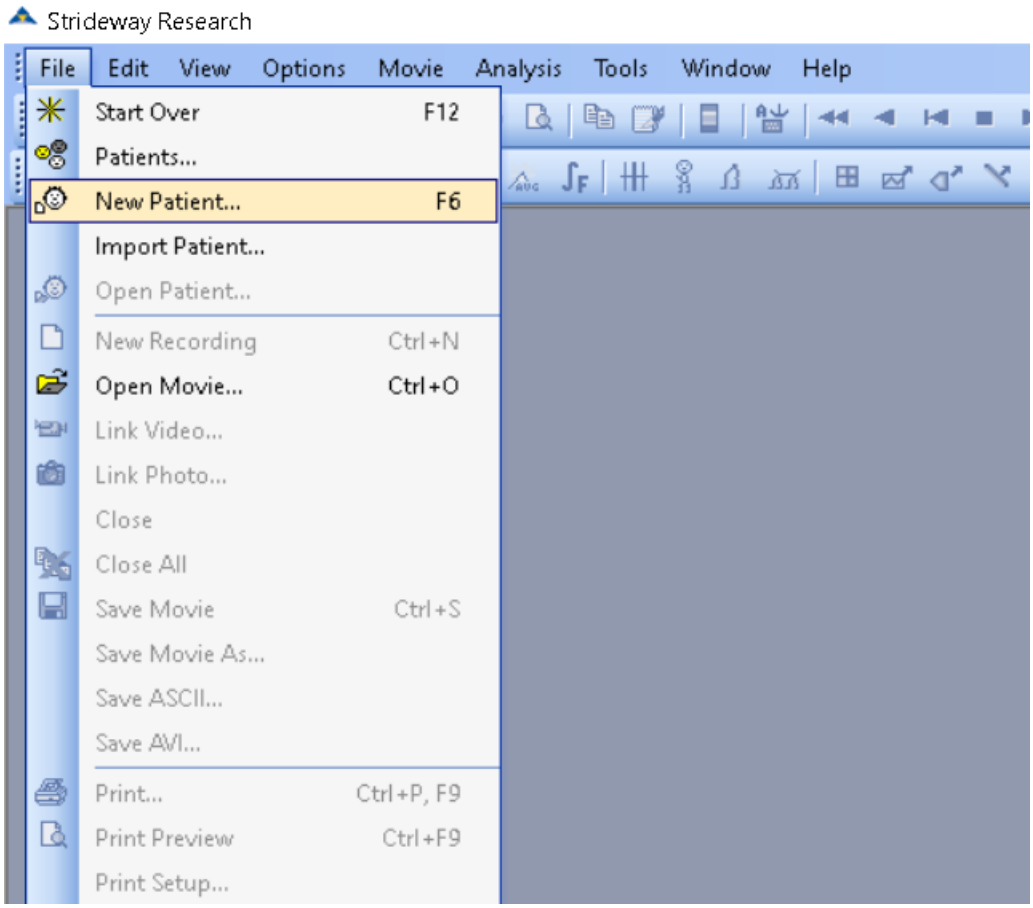


Figure 13 Creation of new patient file

A progressive four digits number must be inserted before the patient surname in the “Last name” box (Figure 14). E.g., if there are already 30 patients saved on the laptop software and the patient surname is Smith (this can be verified checking the patient list) then the new patient surname should be typed as 0031Smith.

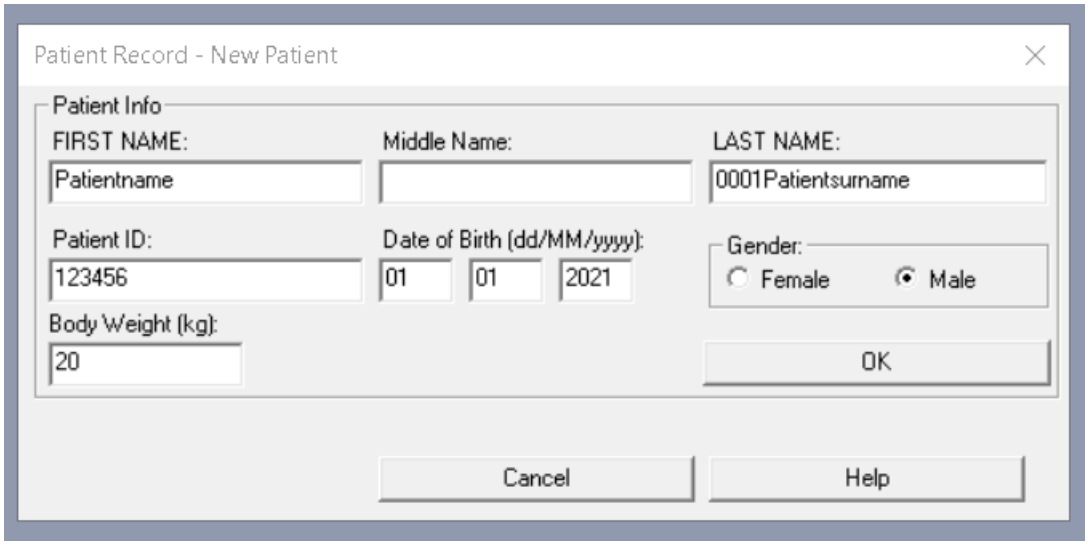


Figure 14 Creation of new patient file, continued

The software will automatically save all patient data in a folder named with the patient's surname. Failing to type the progressive number in front of the surname could cause data from different patients having the same surname to be saved in the same folder and overwrite each other, causing data loss. It is also recommended that new studies of previous patients (e.g., follow up appointments following a treatment) be saved with a new sequential number to avoid data loss.

2. Camera Connection

The camera is activated by clicking "capture video" and selecting Microsoft Camera (Figure 15), which will then link the image from the camera to the pressure mat data. The camera must be positioned so that the field of view always includes the three central active pressure-sensing tiles.

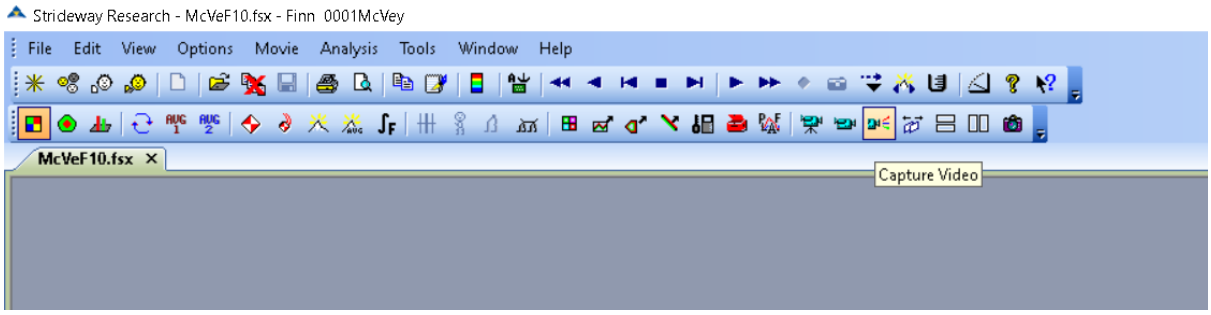


Figure 15 Connection of the camera

3. Selection of walkway sensitivity and system calibration

The next step involves clicking on ”Select Pressure Sensitivity” (Figure 16) and selecting either:

“Load low sensitivity” (usually appropriate for light patients such as cats and small dogs),

“Load medium sensitivity” (usually appropriate for most medium and large size dogs)

“Load high sensitivity” (usually appropriate for very large dogs).

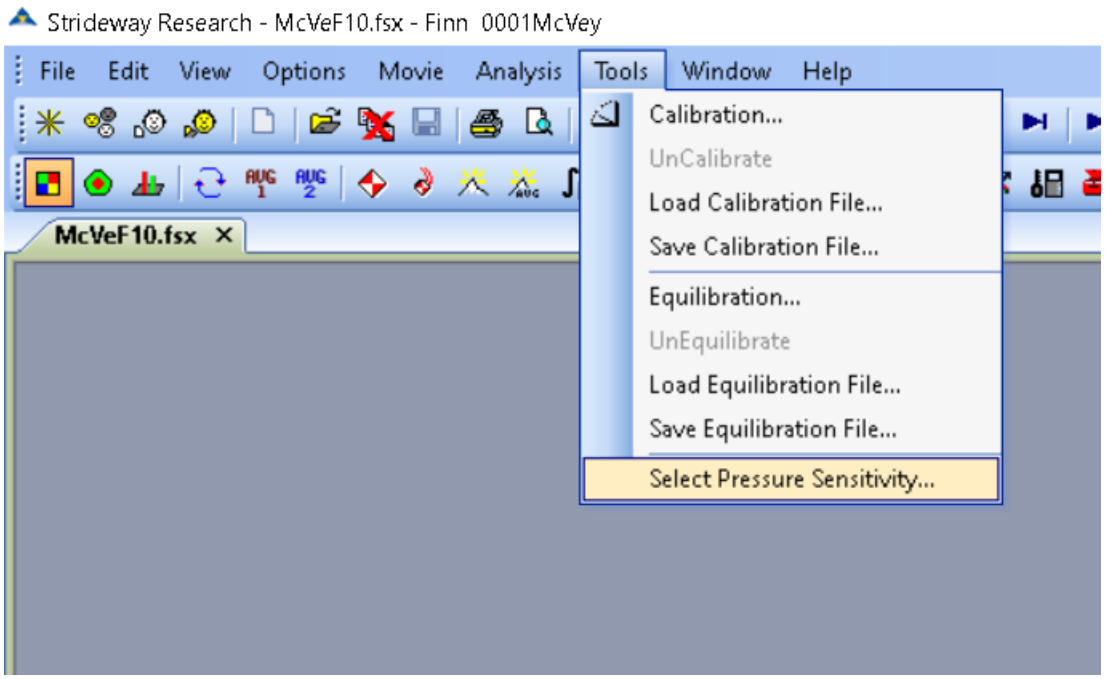


Figure 16 Setting the pressure mat sensitivity

It is important to recognise that the wording chosen by the software developers seems counterintuitive as the **“low sensitivity”** settings are those appropriate for **light** animals. All animals included in this study had data collected using the “medium sensitivity” settings.

The next step is to calibrate the pressure mat, by clicking on “Tools” and selecting “Calibration” (Figure 17). The software will guide the operator through all steps of the calibration process. All data in this study was collected following calibration using the previously described step calibration method using a phantom tool, with sensitivity set at medium.

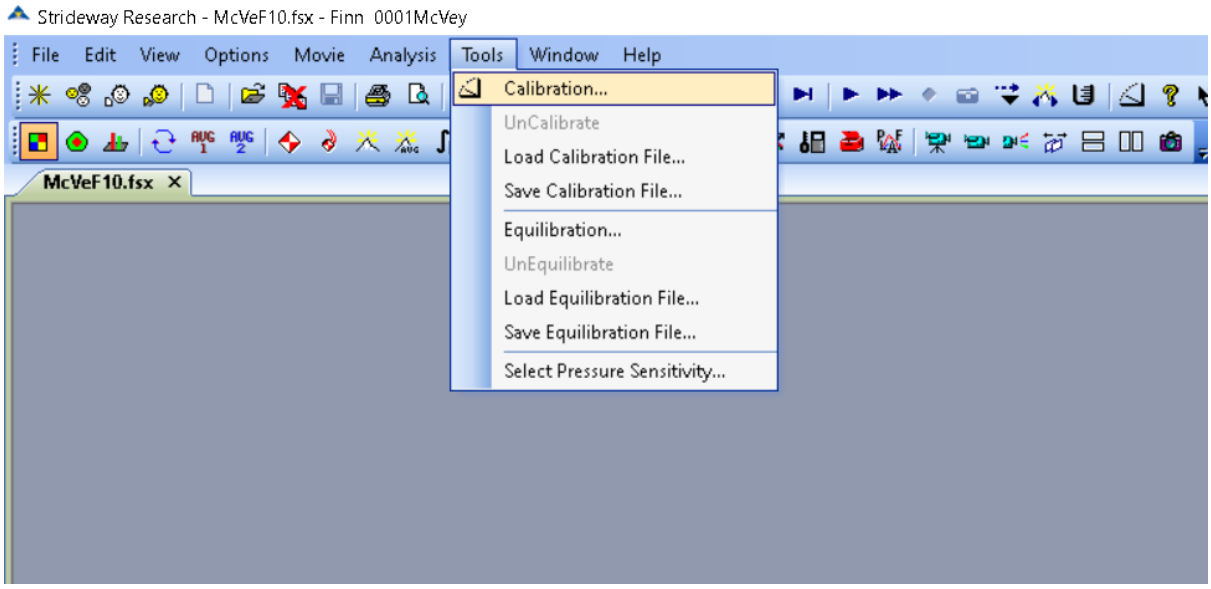


Figure 17 Calibration process

4. Recording patient gait data

In the Tekscan software, gait analysis data acquired by a single pass of the patient over the pressure mat is termed “movie”. To acquire a movie, the red recording button is clicked (Figure 18). This activates the system and recording will be triggered as soon as the patient steps on the mat, and will terminate automatically after the last step. The process is repeated until a sufficient number of satisfactory movies has been recorded (usually at least five acceptable movies).

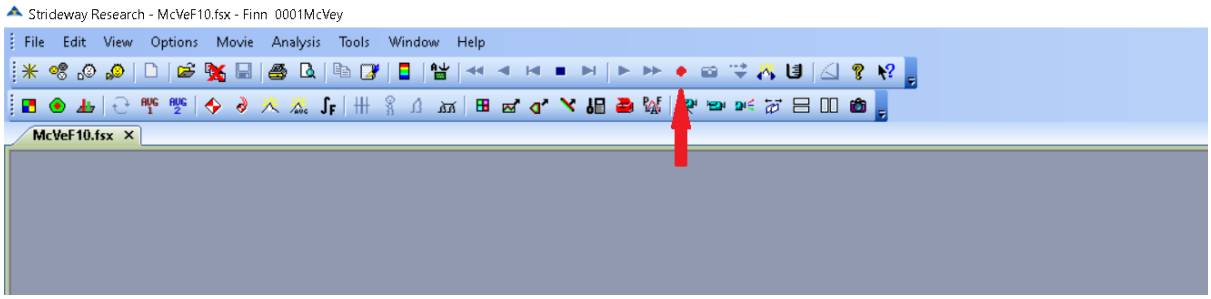


Figure 18 Arrow indicates the button to activate data recording

It is possible to set the system to record multiple passes over the walkway in a single movie. In the authors’ experience this is not recommended as it generates large files with multiple overlapping of steps. This makes the software more likely to crash and the data more difficult to manage and analyse.

If the system has been set properly each movie will comprise of two windows (Figure 19).

The first window shows the pressure generated by each paw at a specific moment in time. It uses a colour code scale where blue indicates a low pressure and red a high pressure (Figure 20).

The second window shows the recorded video of the patient walking over the mat. A time bar and cursor on top of this window enables manual scrolling through the timeline, so that each frame of the recording can be checked. The first and second windows are synchronised.

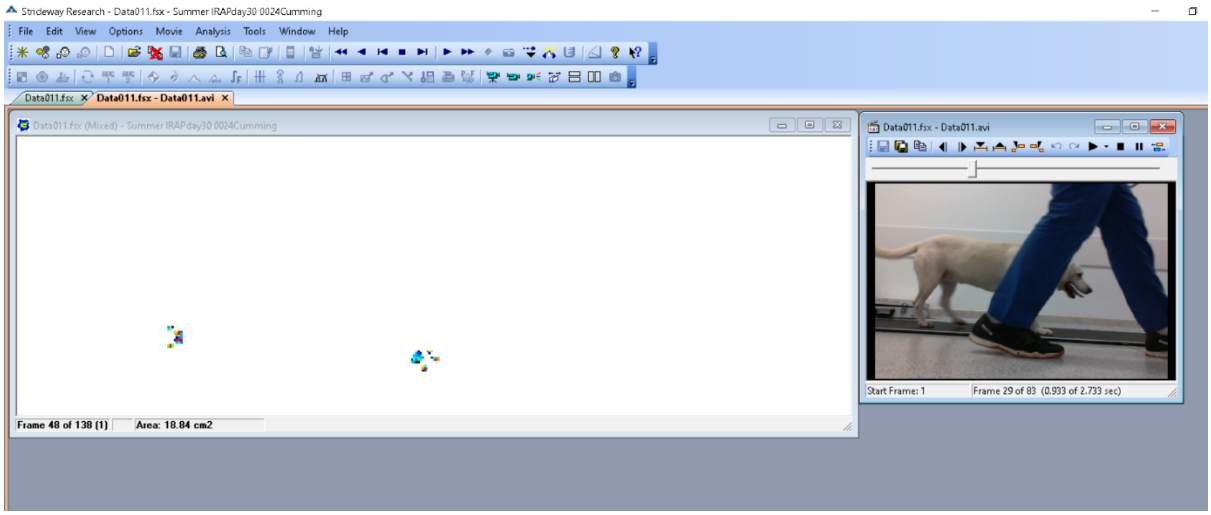


Figure 19 The two windows containing the movie data

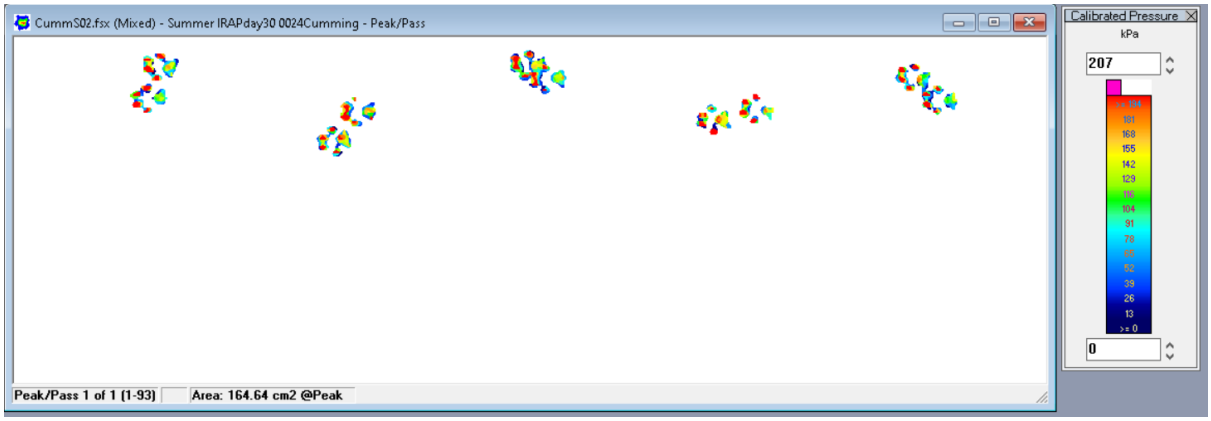


Figure 20 The pressure exerted by each paw shown with a colour code

5. Data analysis

To initiate data analysis, the operator clicks on the “quadrupedal gait” icon (Figure **21**).

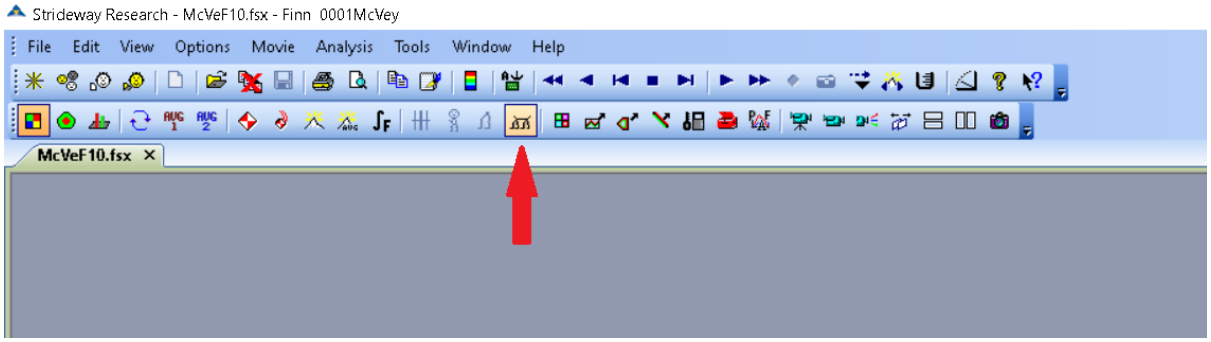


Figure 21 **Arrow indicates the button to activate data analysis**

The software will recognise all paw strikes - a strike is the data generated by a single paw during a single contact with the pressure mat – and automatically draw a box around each strike (hereafter ‘strikeboxes’), and add a label indicating which paw generated the strike (i.e. RF, right forelimb, LF, left forelimb, RH, right hindlimb, LH, left hindlimb).(Figure 22). The software will then ‘ask’ the operator to confirm of the strikeboxes have been correctly labelled before proceeding.

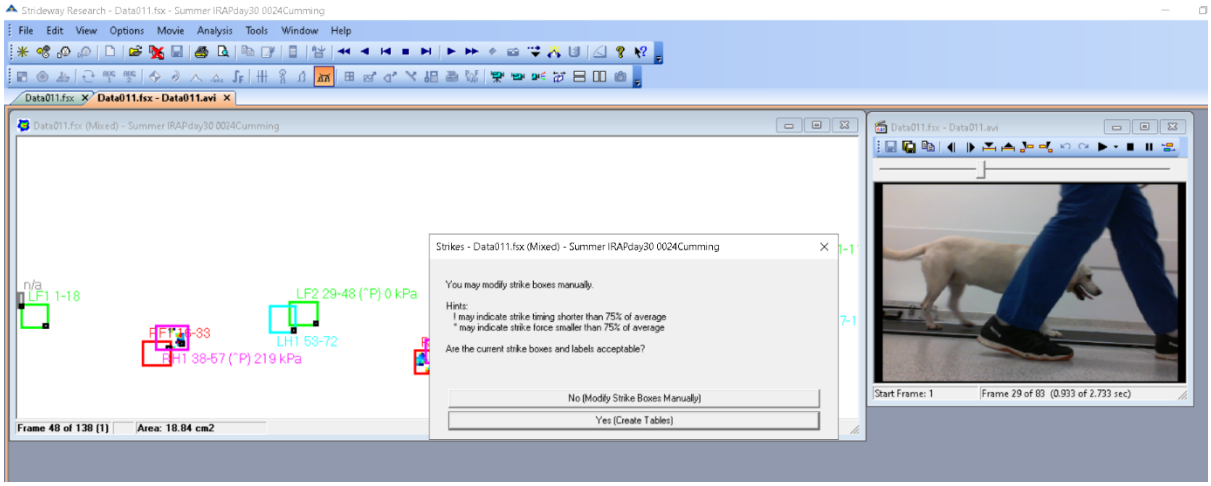


Figure 22 The software asks the operator to check the strikeboxes and labels are acceptable

In the author’s experience, the software either draws inaccurate strikeboxes, or labels them incorrectly, in approximately 5-10% of cases. Therefore all of the stikeboxes and labels have to be manually checked against the video to ensure they are assigned to the correct paw as inaccuracies will alter signficantly the data (e.g. if the low pressure generated by a left hindlimb is interpreted as being generated by the left forelimb) – a very time-consuming process. If for example a strikebox of the right

forelimb has been assigned incorrectly to another limb, it can be easily corrected by right-clicking on the strikebox and selecting “mark as right front” (Figure **23**).

If a paw stike falls on the border of the pressure mat only a portion of it will be detected by the sensels and the data for that will be inaccurate. A strikebox located on the border of the pressure mat should be excluded from analsysis by right-cliking on it and selecting “mark as N/A”.

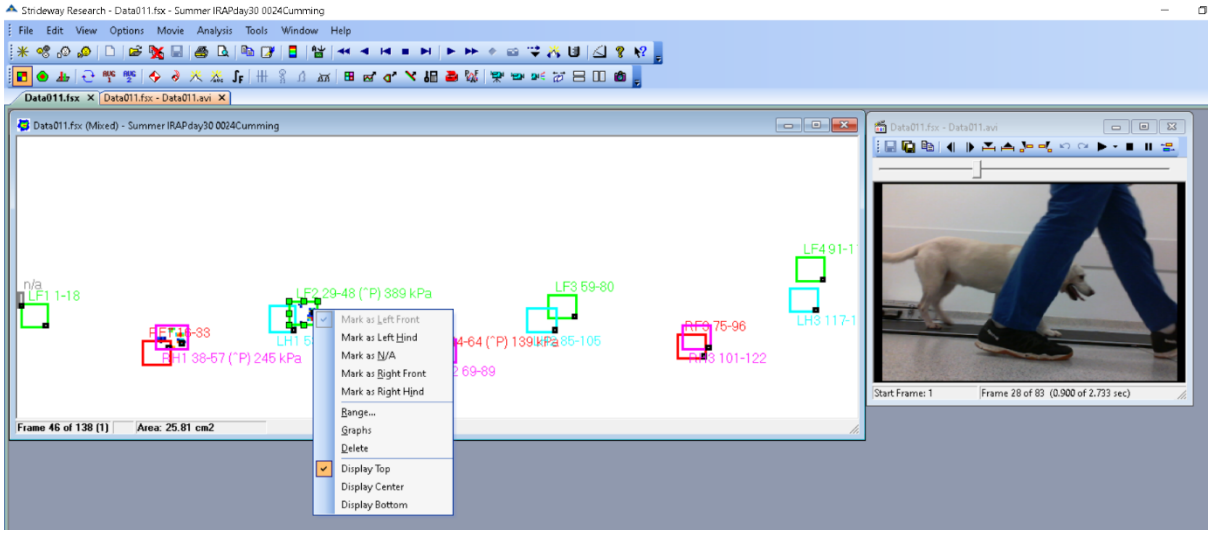
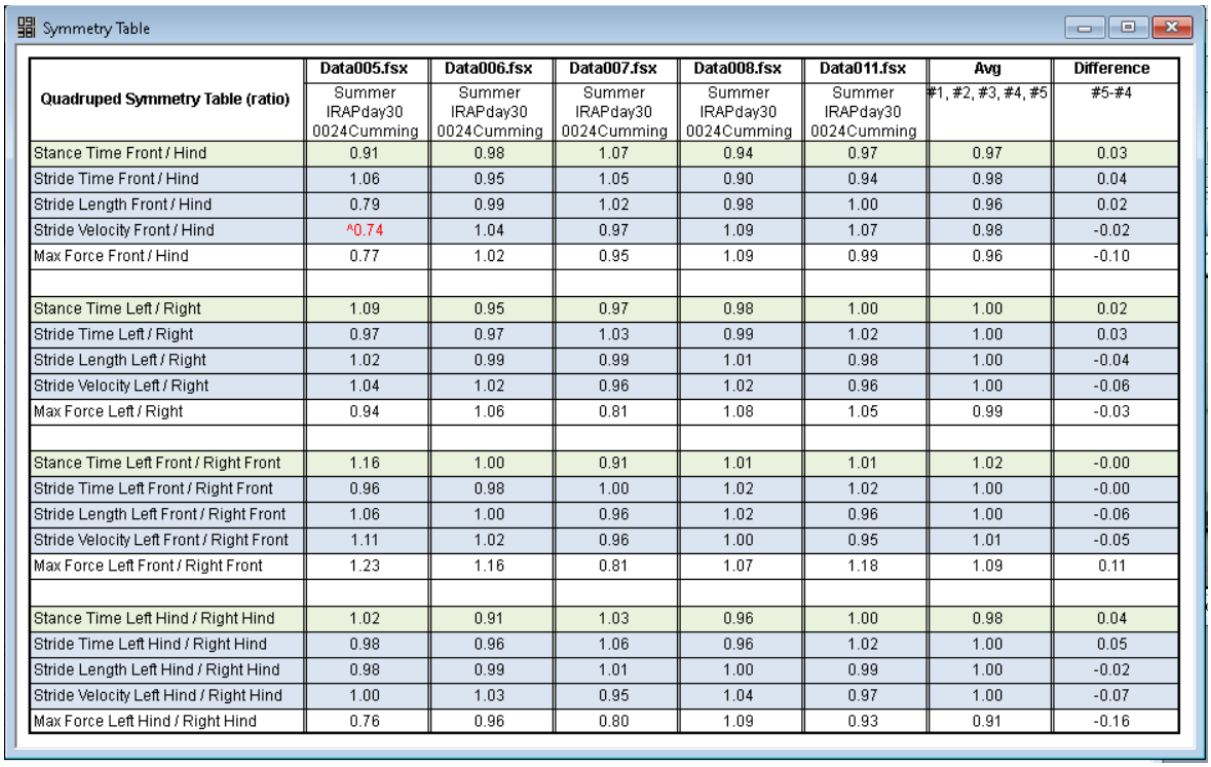


Figure 23 **An incorrectly assigned strike box can be corrected by right clicking on it as select "Mark as ..."**

The software will automatically export all gait analysis data in the form of three tables, as illustrated in the following figures:

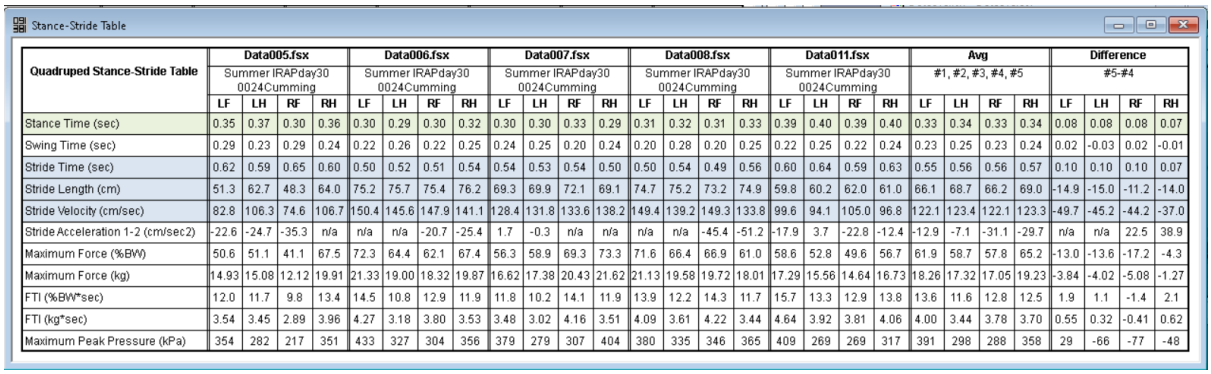
Figure 24 Symmetry table



Symmetry Table

Quadruped Symmetry Table (ratio)	Data005.fsx Summer IRAPday30 0024Cumming	Data006.fsx Summer IRAPday30 0024Cumming	Data007.fsx Summer IRAPday30 0024Cumming	Data008.fsx Summer IRAPday30 0024Cumming	Data011.fsx Summer IRAPday30 0024Cumming	Avg #1, #2, #3, #4, #5	Difference #5-#4
Stance Time Front / Hind	0.91	0.98	1.07	0.94	0.97	0.97	0.03
Stride Time Front / Hind	1.06	0.95	1.05	0.90	0.94	0.98	0.04
Stride Length Front / Hind	0.79	0.99	1.02	0.98	1.00	0.96	0.02
Stride Velocity Front / Hind	^0.74	1.04	0.97	1.09	1.07	0.98	-0.02
Max Force Front / Hind	0.77	1.02	0.95	1.09	0.99	0.96	-0.10
Stance Time Left / Right	1.09	0.95	0.97	0.98	1.00	1.00	0.02
Stride Time Left / Right	0.97	0.97	1.03	0.99	1.02	1.00	0.03
Stride Length Left / Right	1.02	0.99	0.99	1.01	0.98	1.00	-0.04
Stride Velocity Left / Right	1.04	1.02	0.96	1.02	0.96	1.00	-0.06
Max Force Left / Right	0.94	1.06	0.81	1.08	1.05	0.99	-0.03
Stance Time Left Front / Right Front	1.16	1.00	0.91	1.01	1.01	1.02	-0.00
Stride Time Left Front / Right Front	0.96	0.98	1.00	1.02	1.02	1.00	-0.00
Stride Length Left Front / Right Front	1.06	1.00	0.96	1.02	0.96	1.00	-0.06
Stride Velocity Left Front / Right Front	1.11	1.02	0.96	1.00	0.95	1.01	-0.05
Max Force Left Front / Right Front	1.23	1.16	0.81	1.07	1.18	1.09	0.11
Stance Time Left Hind / Right Hind	1.02	0.91	1.03	0.96	1.00	0.98	0.04
Stride Time Left Hind / Right Hind	0.98	0.96	1.06	0.96	1.02	1.00	0.05
Stride Length Left Hind / Right Hind	0.98	0.99	1.01	1.00	0.99	1.00	-0.02
Stride Velocity Left Hind / Right Hind	1.00	1.03	0.95	1.04	0.97	1.00	-0.07
Max Force Left Hind / Right Hind	0.76	0.96	0.80	1.09	0.93	0.91	-0.16

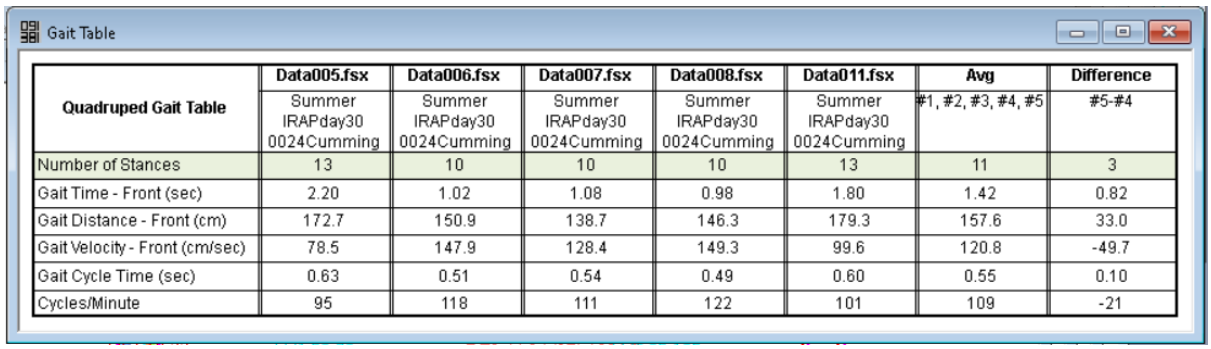
Figure 25 Stance-Stride table



Stance-Stride Table

Quadruped Stance-Stride Table	Data005.fsx Summer IRAPday30 0024Cumming				Data006.fsx Summer IRAPday30 0024Cumming				Data007.fsx Summer IRAPday30 0024Cumming				Data008.fsx Summer IRAPday30 0024Cumming				Data011.fsx Summer IRAPday30 0024Cumming				Avg #1, #2, #3, #4, #5				Difference #5-#4			
	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH
Stance Time (sec)	0.35	0.37	0.30	0.36	0.30	0.29	0.30	0.32	0.30	0.30	0.33	0.29	0.31	0.32	0.31	0.33	0.39	0.40	0.39	0.40	0.33	0.34	0.33	0.34	0.08	0.08	0.08	0.07
Swing Time (sec)	0.29	0.23	0.29	0.24	0.22	0.26	0.22	0.25	0.24	0.25	0.20	0.24	0.20	0.28	0.20	0.25	0.22	0.25	0.22	0.24	0.23	0.25	0.23	0.24	0.02	-0.03	0.02	-0.01
Stride Time (sec)	0.62	0.59	0.65	0.60	0.50	0.52	0.51	0.54	0.54	0.53	0.54	0.50	0.50	0.54	0.49	0.56	0.60	0.64	0.59	0.63	0.55	0.56	0.56	0.57	0.10	0.10	0.10	0.07
Stride Length (cm)	51.3	62.7	48.3	64.0	75.2	75.7	75.4	76.2	69.3	69.9	72.1	69.1	74.7	75.2	73.2	74.9	59.8	60.2	62.0	61.0	66.1	68.7	66.2	69.0	-14.9	-15.0	-11.2	-14.0
Stride Velocity (cm/sec)	82.8	106.3	74.6	106.7	150.4	145.6	147.9	141.1	128.4	131.8	133.6	138.2	149.4	139.2	149.3	133.8	99.6	94.1	105.0	96.8	122.1	123.4	122.1	123.3	-49.7	-45.2	-44.2	-37.0
Stride Acceleration 1-2 (cm/sec2)	-22.6	-24.7	-35.3	n/a	n/a	n/a	-20.7	-25.4	1.7	-0.3	n/a	n/a	n/a	n/a	-45.4	-51.2	-17.9	3.7	-22.8	-12.4	-12.9	-7.1	-31.1	-29.7	n/a	n/a	22.5	38.9
Maximum Force (%BW)	50.6	51.1	41.1	67.5	72.3	64.4	62.1	67.4	56.3	58.9	69.3	73.3	71.6	66.4	66.9	61.0	58.6	52.8	49.6	56.7	61.9	58.7	57.8	65.2	-13.0	-13.6	-17.2	-4.3
Maximum Force (kg)	14.93	15.08	12.12	19.91	21.33	19.00	18.32	19.87	16.62	17.38	20.43	21.62	21.13	19.58	19.72	18.01	17.29	15.56	14.64	16.73	18.26	17.32	17.05	19.23	-3.84	-4.02	-5.08	-1.27
FTI (%BW*sec)	12.0	11.7	9.8	13.4	14.5	10.8	12.9	11.9	11.8	10.2	14.1	11.9	13.9	12.2	14.3	11.7	15.7	13.3	12.9	13.8	13.6	11.6	12.8	12.5	1.9	1.1	-1.4	2.1
FTI (kg*sec)	3.54	3.45	2.89	3.96	4.27	3.18	3.80	3.53	3.48	3.02	4.16	3.51	4.09	3.61	4.22	3.44	4.64	3.92	3.81	4.06	4.00	3.44	3.78	3.70	0.55	0.32	-0.41	0.62
Maximum Peak Pressure (kPa)	354	282	217	351	433	327	304	356	379	279	307	404	380	335	346	365	409	269	269	317	391	298	288	358	29	-66	-77	-48

Figure 26 Gait table



Gait Table

Quadruped Gait Table	Data005.fsx Summer IRAPday30 0024Cumming	Data006.fsx Summer IRAPday30 0024Cumming	Data007.fsx Summer IRAPday30 0024Cumming	Data008.fsx Summer IRAPday30 0024Cumming	Data011.fsx Summer IRAPday30 0024Cumming	Avg #1, #2, #3, #4, #5	Difference #5-#4
Number of Stances	13	10	10	10	13	11	3
Gait Time - Front (sec)	2.20	1.02	1.08	0.98	1.80	1.42	0.82
Gait Distance - Front (cm)	172.7	150.9	138.7	146.3	179.3	157.6	33.0
Gait Velocity - Front (cm/sec)	78.5	147.9	128.4	149.3	99.6	120.8	-49.7
Gait Cycle Time (sec)	0.63	0.51	0.54	0.49	0.60	0.55	0.10
Cycles/Minute	95	118	111	122	101	109	-21

The data from these tables can be easily exported to Microsoft Excel by right clicking on the table and selecting “copy” and then clicking “paste” over a new Excel spreadsheet.

Outcome measures obtained from the pressure walkway and used for analysis were:

- PVF % = peak vertical force normalised for bodyweight, named “Maximum Force (%BW)” in Figure 25
- Vi% = vertical impulse normalised for bodyweight, named “FTI (%BW*sec)” in Figure 25
- SI R/L = symmetry index for right forelimb/left forelimb, named “Max Force Right front/Left front in Figure 24. This value was used for analysis only in dogs that received treatment in the right elbow.
- SI L/R = symmetry index for left forelimb/right forelimb. This value is manually calculated with the formula “1 / (SI R/L)”. This value was used for analysis only in dogs that received treatment in the left elbow.
- SI F/H = symmetry index for forequarters/hindquarters. Named “Max force Front/Hind” in Figure 24.

2.3.7 Pressure sensitive walkway data acquisition protocol

1. The dog is allowed to acclimatise in the area of the PSW (Figure 1211). While kept on a lead, the patient is allowed to freely walk across the pressure mat for 5 minutes.
2. The patient is walked on the pressure mat as he would during a valid trial for 5-10 times until he appears to have familiarised with the device (i.e. it does not try to stop or walk off the walkway)
3. 20 trials are recorded with the handler alternatively on the right and left respectively side of the patient. Valid movies are those where:

    - The patient moves at a self-selected but steady pace, either walking or trotting. Acceptable ranges were defined as: 1.0-1.4 m/s with acceleration of ± 0.5 m/s^2 at the walk, and 1.7 to 2.1 m/s with acceleration of ± 0.5 m/s^2 at the trot.

- The patient moves in a straight line, with the head pointing forward, without obviously looking around or turning sideways
- The lead appears loose throughout the duration of the movie.

4 Subsequently (this is usually performed at later stage) all acquired movies are reviewed and the 5 most appropriate, based on the above criteria are selected for analysis.

2.4 VON FREY

The electronic VonFrey anaesthesiometer (IITC, II-2391, World Precision Instruments, UK) used during this project consisted of a hand-held applicator with a plastic tip, a load cell connected to a recording device and a hand-held reset button. The load cells can measure a maximum of 800g and are accurate to 0.1g.

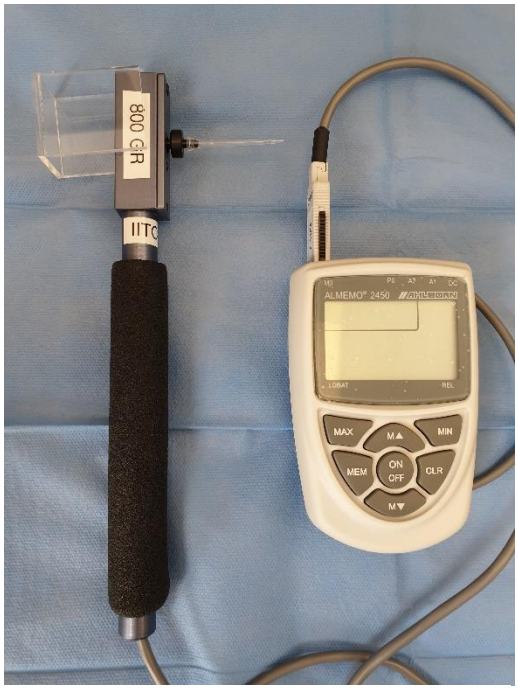


Figure 28 VonFrey hand held applicator (left) and recording device (right)

Figure 27 Von Frey applied against the carpal pad

2.4.1 Device set-up

1.

The hand-held applicator, the hand-held reset button and power supply are connected to the recording device. The plugs on the recording device (Figure 30) and the respective connectors (Figure 29) are clearly labelled and designed in a way that prevents incorrect connections.

Figure 29 Connectors of applicator (MO), reset button (black A1/A2) and power supply (white A1/A2)

Figure 30 Plugs on the recording device

2.

The device is calibrated by pressing the CLR button (i.e. pressing CLR sets the device to 0 grams). Device accuracy needs to be verified by placing the provided 5.1g weight on the cell and ensuring the weight is assessed correctly (Figure 31).

Figure 31 The provided 5.1g weight (left), calibrated device with no load (centre), device correctly measuring 5.1g after weight is placed on the cell

3.

The plastic tip is applied on to the load cell (Figure 32)

Figure 32 Hand-held applicator with plastic tip applied

4.

The button MAX is pressed (Figure 28). This instructs the device to record the maximum weight (in grams) applied against the tip, which is shown on the top left corner of the screen.

2.4.2 Von Frey data acquisition protocol:

- Test to be performed in a quiet environment. Before beginning the test, the patient was given 10 minutes off the lead to relax in the room (this time was used to prepare and calibrate the device).
- One assistant gently restrained the patient while the operator performed the test. The Von Frey screen should be visible only to the assistant and the operator should be blinded of the value.
- With the patient standing the operators pointed the tip of the device on the middle of the carpal pad. A progressive force was applied to the device until the patient reacted (withdrawal of the paw, escape movement, vocalization) OR 400g of pressure was reached. As the operator should was not allowed to see the measured value, the assistant advised if the limit of 400g of pressure was reached. Withdrawal of the paw at first light contact with the von Frey was not considered a valid trial.
- Five valid reading in each carpal pad were performed. Left and right measurement were alternated (so that if patient reaction changed with time or with patient getting used to the test this did not bias the results). Between each measurement the device was reset by pressing the handheld reset button or alternatively the CLR button (Figure 33).

Figure 33 Hand-held button (left) and recording device (right). The device is showing that a maximum pressure of 34.9g has been applied

The outcome measures obtained from the Von Frey Device were recorded as:

Von Frey right leg = the average (in grams) of five Von Frey measurements performed on the right forelimb.

Von Frey left leg = the average (in grams) of five Von Frey measurements performed on the tight forelimb.

Further details on the protocol used for von Frey testing in this project are contained in Appendix 4.

2.5 LOAD QUESTIONNAIRE

The Load questionnaire is a client-completed validated method of assessing the effect of canine osteoarthritis on the dog's overall mobility, comfort and quality of life. It contains sections assessing background, lifestyle and mobility (Walton *et al.*, 2013). The questionnaire was given to clients at every visit and is available in two versions:

- The "Initial Visit" version consists of 3 "Background" questions, 7 "Lifestyle" questions and 13 "mobility" multiple choice questions.
- The "Follow Up Visit" version consists of only the 13 "mobility" multiple choice questions.

Clients were given privacy in a consult room with unlimited time to fill the questionnaire, and they were free to go at any time, leaving the filled questionnaire at the reception desk.

Each of the 13 mobility questions has 5 possible answers (see Figure 34), that will generate a score from 0 (for the more positive answer) to 4 (for the more negative answer). The scores from each of the 13 questions are then added up to generate a final score ranging from 0 (patients are completely healthy) to 52 (for patients with very severely affected mobility). In both versions of the questionnaire only the "mobility" questions are utilised to calculate the LOAD score which relates to the patient function and response to treatment. Answers in the background and lifestyle sections are recorded but do not contribute to the final score. (Note: the LOAD score is the only outcome measure utilised in this study where a *decreasing* value indicates an *improvement*).

Figure 34 Example of a LOAD question – selecting "very poor" generate a score of 4

2.6 V-PET

2.6.1 Description of the kit

V-Pet (Pall Corporation, New York, UK) is a commercially available point of care kit. Kits for this study were donated by VBS Direct (Whitchurch, UK).

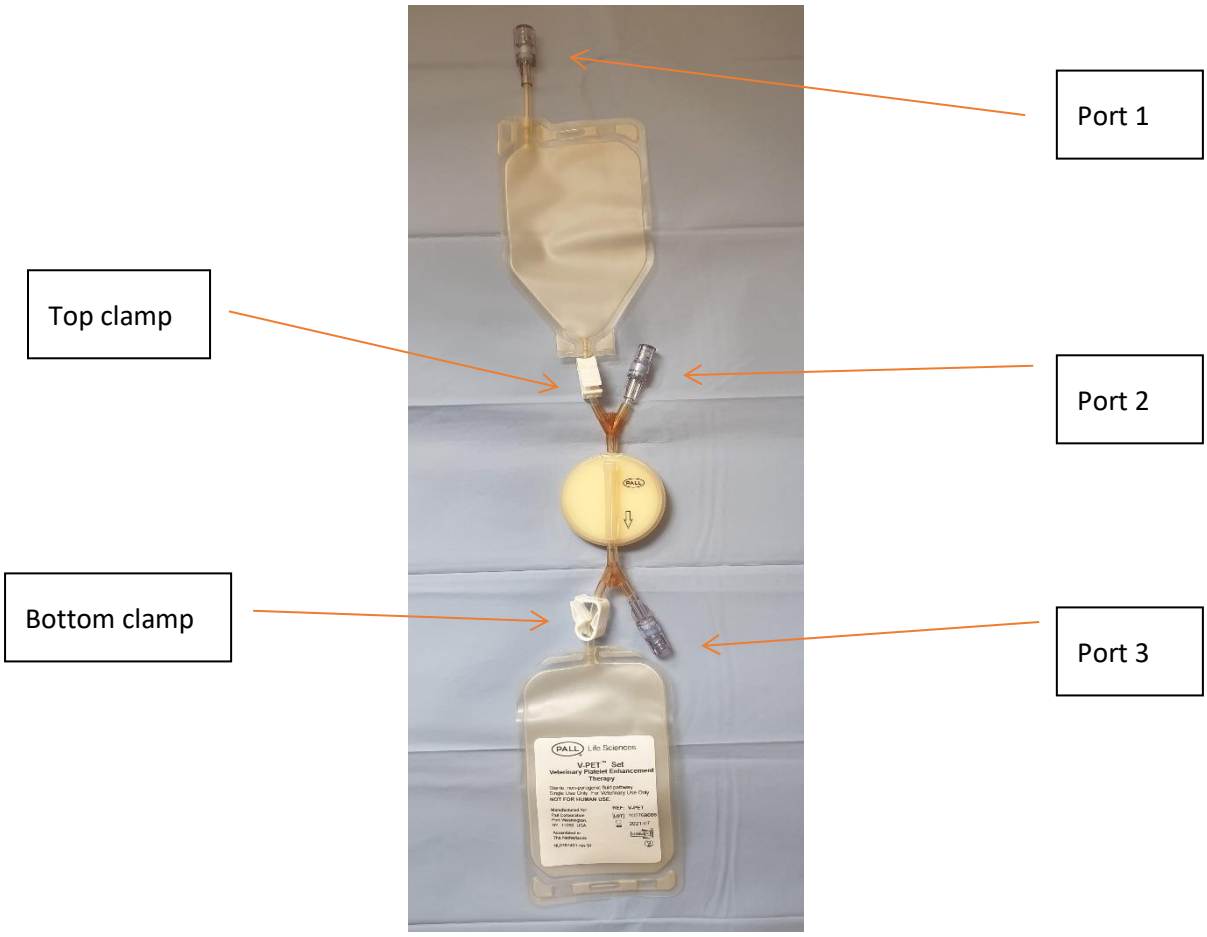


Figure 35 Filtration system with the three ports and two clamps, the bag on top of the figure, is attached to the drip stand.

The main component of the kit is the "filter system" which is provided sterile in a sealed bag. The filter system is made of two empty plastic fluid bags (top bag and bottom bag) which are connected by a patented filter. One clamp is placed at the top of the filter (top clamp) and one at the bottom of the filter (bottom clamp) to control the flow of fluids in the system. Three ports located in the top bag (Port1), on the top part of the filter (port2) and bottom part of the filter (Port3) allow introduction of fluids into the system and retrieval of the platelet concentrate (Figure 35).

The system must be kept in a vertical position during use and is designed to be hung from a drip stand; the top bag must be positioned higher than the bottom bag. An arrow is printed on the filter, and must always point downwards, indicating the correct direction of fluid flow from the top bag towards the bottom bag.

Additionally, the kit contains three 10ml syringes, one 60ml syringe, a 20ml vial of water for injection (solution A), a 20ml vial of sodium citrate (solution B), a 20ml vial of hypertonic 2% NaCl solution (solution C), a sterile drape, and Instructions for use (

Figure 3636).

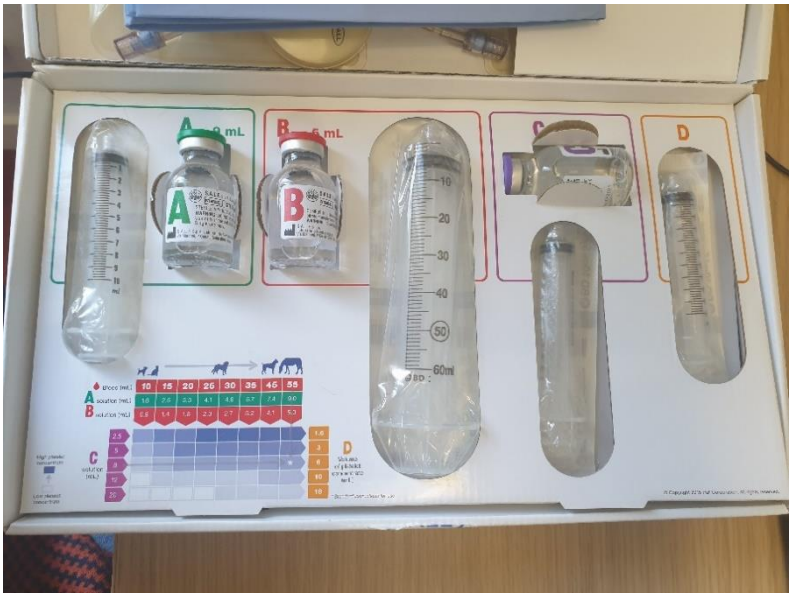


Figure 36 Content of the V-PET kit containing solutions A (green lid), B (red lid) and C (purple lid). The filtration system is shown in Figure 35)

2.6.2 Platelet concentrate preparation

The following protocol was used in every case: following intravenous access was secured by placing an intravenous catheter into one of the cephalic veins. When possible, the forelimb that was least affected by elbow osteoarthritis was used for placement of the intravenous catheter, alternatively a saphenous vein was catheterised. The patient was then sedated with a combination of 0.01mg/kg medetomidine (Domitor, Vetquinol) and 0.1mg/kg butorphanol (Torbugesic, Zoetis) intravenously. With the patient in lateral recumbency an area over the jugular vein of approximately 10cm x 10cm was clipped and prepped using a combination of chlorhexidine and isopropyl alcohol (Chloraprep, BD).

One operator set up the kit following the manufacturer instructions and wearing sterile gloves. The filter system was hung on a drip stand and both the top and bottom clamps were closed. Nine millilitres of water for injection (solution A) were injected into the top bag via Port1.

Next, 5 ml of sodium citrate (solution B) were loaded into the 60ml syringe and an 18-gauge butterfly needle was connected the same syringe. Always wearing sterile gloves, the 60ml syringe and butterfly needle were then used to collect 55ml of blood from the jugular vein, which mixed with the 5mls of anticoagulant, to produce a total volume of 60mls of anticoagulated blood. The anticoagulated blood was then injected into the top bag via port 1 and gently mixed with the 9mls of water for injection that had been previously injected into the same bag.

Next, both the top clamp and bottom clamp were then opened allowing the anticoagulated blood to flow by gravity from the top bag through the filter and into the bottom bag. As the anticoagulated blood flows through the patented filter, platelets and (in lower numbers) white blood cells are selectively trapped by the filter while red blood cells and serum are allowed to flow into the bottom bag. The entire process of filtration takes approximately 15-20minutes.

When all the anticoagulated blood has passed into the bottom bag, a 10ml syringe filled with 8mls of hypertonic saline (solution C) is firmly connected to Port 3 an empty 10ml syringe is connected to Port 2. Next, both the top clamp and the bottom clamp are closed again, in this way isolating the filter, port 2 and port 3 from both bags. The 8 ml of solution C are then flushed through port 3, passing 'retrograde' through the filter, and collecting in the empty syringe connected to port 2. (Figure 38) During the flushing, platelets that were contained in the filter are dislodged and collected in the

Figure 37 V-PET kit during filtration process (Adapted from www.vbsdirect.co.uk)

syringe connected to port 2. Approximately 2ml of solution are lost into the dead space of the system, resulting on average in 6ml of what is then termed “platelet concentrate” being collected.

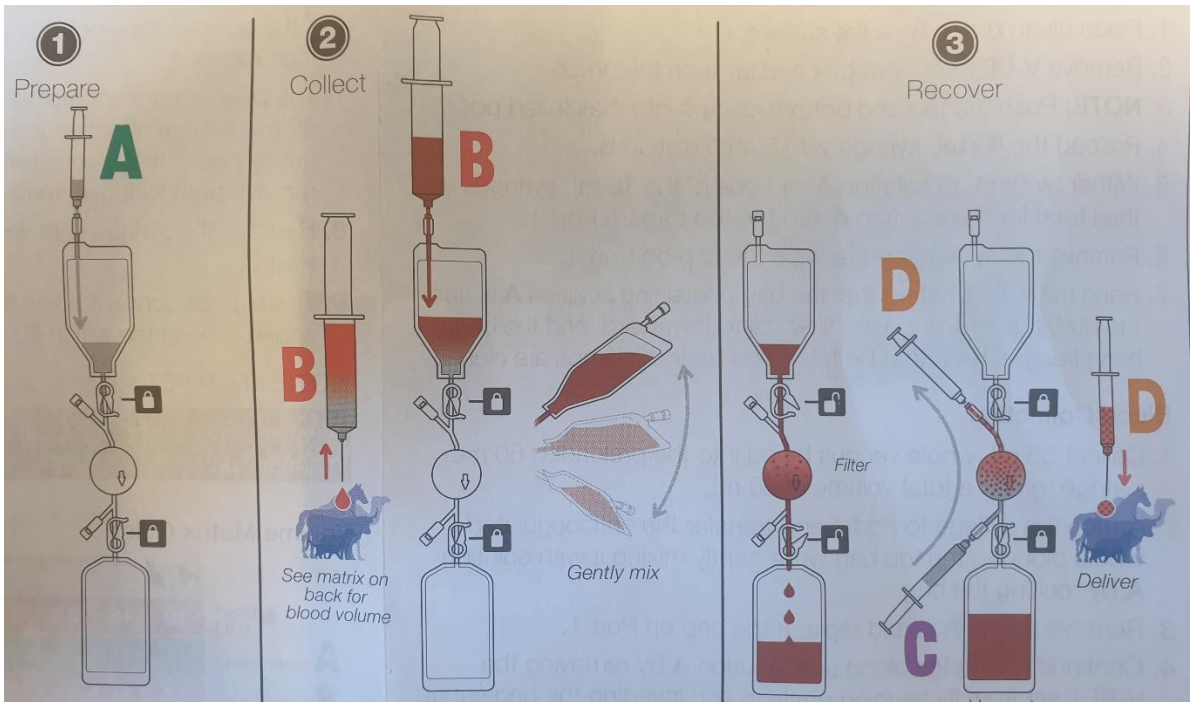


Figure 38 VPET leaflet, schematic showing steps for preparation of the platelet concentrate

2.6.3 Sample management and administration

Following principles of asepsis, wearing sterile gloves, the 6ml of platelet concentrate was divided into two sterile aliquots of 3ml each. Patients in this study received a single intraarticular injection of platelet concentrate, and the second dose was labelled with patient details and date and stored for analysis and quality control purposes. When this kit is used in clinical settings the second aliquot can be stored at -18 degrees Celsius for up to a year, and be used for a second intraarticular treatment.

Under the same sedation used for blood sampling the patient was placed in lateral recumbency with the affected elbow uppermost. An area of approximately 5cm x 5cm of skin over the lateral aspect of

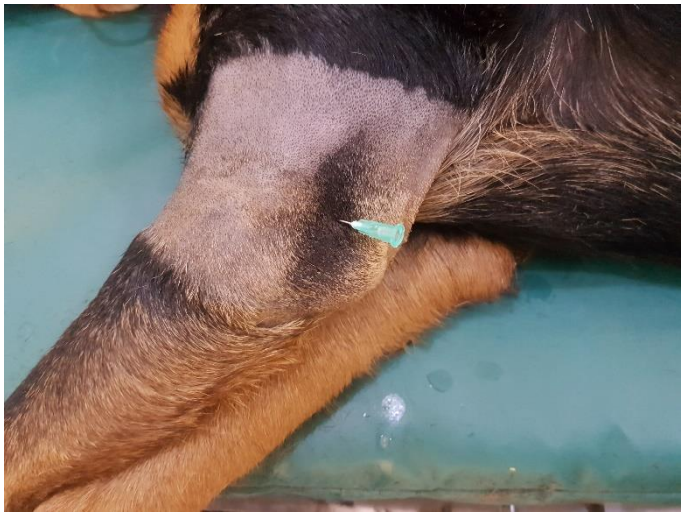


Figure 39 Arthrocentesis needle correctly placed

the olecranon was clipped and prepped with a combination of chlorhexidine and isopropyl alcohol (Chloraprep).

Using a sterile technique, elbow arthrocentesis was performed with a 1.5inches 21gauge needle and a 5ml syringe. Successful positioning within the joint space was confirmed by retrieval of synovial fluid. Holding the needle in place, the syringe is then disconnected, and the syringe containing the platelet concentrate connected, and the platelet concentrate produce slowly injected into the joint space. The injection was stopped as soon as resistance was felt (i.e., the syringe plunger being pushed back by increased intraarticular pressure); on average, 2.5ml of platelet concentrate were administered to each patient. The needle was then removed and the elbow flexed and extended 10 times to help the platelet concentrate to distribute throughout the joint space. Sedation was then reversed with 0.05mg/kg of atipamezole (Antisedan, Vetquinol) via intramuscular injection to antagonise the effect of medetomidine.

The patient was then kept in hospital for monitoring for approximately 12 hours after the injection. Buprenorphine (Vetergesic, Ceva) 0.02mg/kg intravenously, was administered 2 hours after recovery from the sedation and subsequently every 6 hours until discharge.

2.7 ORTHOKINE

2.7.1 Description of the kit

Orthokine vet irap is a commercially available point of care kit for the production of autologous conditioned sera (ACS) in veterinary patients. Kits utilised in this study were kindly donated by Orthogen (Dusseldorf, Germany).

Each kit consists of two individually packed 10ml Orthokine syringes, each containing approximately 20 small (approximately 2mm diameter) patented glass beads. The patented glass beads act to stimulate cytokine and growth factor release from leukocytes in whole blood – these are then concentrated to produce the ACS. Additionally, a 0.22µm filter unit, an 18g butterfly needle and luer adaptor, a 20g 70mm spinal needle and a sterile luer cap are required and are provided separately (Figure 40).

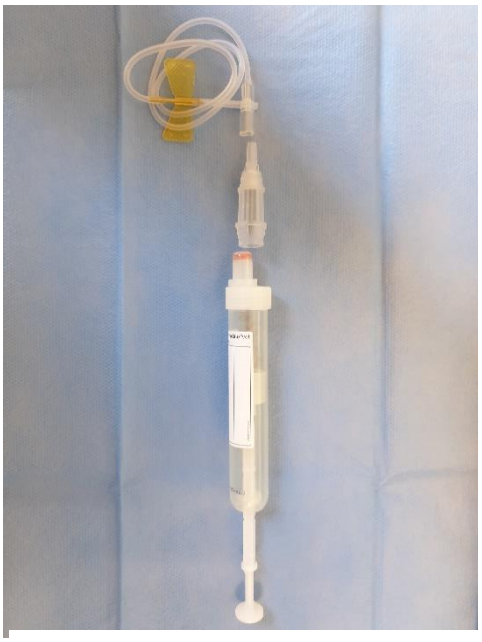


Figure 40 Orthokine Syringe, luer lock adapter, butterfly needle

2.7.2 Autologous conditioned serum preparation

The patient was gently manually restrained in a sitting position and a 10 x 10cm area over the jugular vein prepped as before (see section 2.6.2).

Using aseptic technique and wearing sterile gloves, the first Orthokine syringe was connected to the luer adaptor and to the butterfly needle. The needle was advanced into the jugular vein and the first

syringe filled with 10mls of blood. Without dislodging the needle from the vein, the first syringe was disconnected from the luer adaptor and a second Orthokine syringe was connected and also filled with 10mls of blood. Both Orthokine syringes were gently agitated to allow the glass beads to mix with the blood. The syringes were then labelled with the patient details and placed in an incubator at 37 degrees Celsius for 6 to 9 hours. During this period the patient was returned to a kennel and allowed to eat and rest.

Following incubation, the syringes were centrifuged at 3000RCF (relative centrifugal force, corresponding to 6000 revolutions per minute in our centrifuge) for 10 minutes, as per the kit manufacturer's instructions, to separate the serum. The Orthokine syringes were immediately carefully removed from the centrifuge and placed into a rack on a disinfected working surface. The red stopper septum on each syringe cap was disinfected using a 2% chlorhexidine, 70% alcohol wipe and perforated with a sterile 70mm long, 20 g needle connected to a 5 ml syringe to aspirate the supernatant autologous conditioned sera (Figure 41). Two doses of approximately 3ml each were normally obtained (Figure 43). Both syringes were labelled with the patients details and date. The first aliquot was stored at 4°C and used with 24 hours, while the second dose was stored at -18°C and administered one week after the first dose.

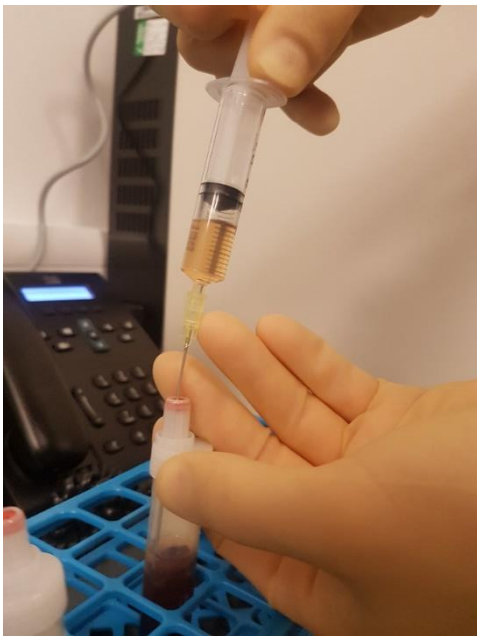


Figure 41 Supernatant autologous conditioned sera being aspirated

2.7.3 Sample management and administration

Patients included in this research project received two intraarticular injections of autologous conditioned sera one week apart as per the manufacturer's recommendation. Blood sampling was always performed in the morning, so that due to the required incubation time of 6 to 9 hours, the product was available by the late afternoon/evening. For this reason, one dose was stored in the fridge at 4°C to be used in the morning of the following day to avoid sedating a patient when less staff was available to monitor it. The second dose was stored in the freezer at -18°C to be used one week later.

Patients were sedated with the same protocol described for the VPET group (see 2.6.2). With the patient in lateral recumbency an area of approximately 5cm x 5cm of skin over the lateral aspect of the olecranon was clipped and prepped, and arthrocentesis performed, as previously described (see section 2.6.2, and Figure 38). The syringe containing the ACS is attached to the filter (0.22um, PES-membrane, Figure 42) which purpose is to trap any bacteria that might have develop during the incubation period. The syringe used to aspirate the synovial fluid is then carefully disconnected from the needle in the joint, and the filter+ACS syringe connected. The ACS is then gently injected into the joint space until resistance was encountered; approximately 2.5ml of ACS were administered in each patient. Thereafter the same protocol of gentle elbow manipulation, reversal of sedation, monitoring and analgesia was followed as with the V-PET cases.

Figure 43 ACS aliquot ready for administration or storage

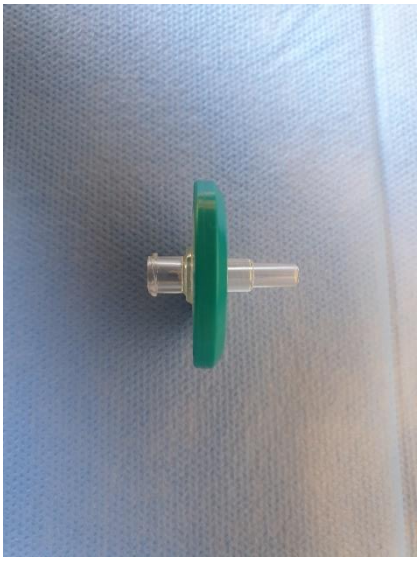


Figure 42 PES membrane filter

2.8 PROTOCOL FOR TREATING CLINICAL CASES

Recruitment:

Clinical cases were recruited among patients that had been previously referred to the Orthopaedic Service. Additionally veterinary general practices in the area were contacted by email and asked if they had patients that could benefit from enrolment in the trial and fit the inclusion criteria.

The author phoned all clients, prior to the first appointment, and discussed in detail the study protocol which made easier for the client to give informed consent at the time of the appointment.

Day 0, Treatment:

- Client was greeted, and study design was briefly discussed again, giving client opportunity to ask questions, and going through the "Project information sheet" (Appendix 1)
- Patient history was obtained and a complete clinical examination performed. (See Appendix 2)
- Client was to sign study consent form (Appendix 3) and also a standard clinical consent form. The latter stated "Hospitalise for sedation and intra-articular injection in LEFT/RIGHT elbow. Risks: related to sedation/anaesthetic, temporary worsening of the lameness, joint infection"
- Client was asked to fill LOAD questionnaire "initial visit" (the questionnaire is different for follow-up)
- Patient was hospitalised
- Pressure mat analysis was performed, as described in Chapter 2.3.7.
- Von Frey test was performed, as described in Appendix 4.
- Patient was assigned to treatment group (V-PET or Orthokine). To allow the operator to familiarise and become proficient with the use of each kit the first 4 patients were allocated to the V-PET group, and the following 4 to the Orthokine group. Subsequently, patients were randomly allocated by the flip of a coin.
- In-house blood tests were performed, unless patient had blood tests performed within 3 months. If blood test results suggested sedation is not safe, patient was excluded from the study. If patient was thrombocytopenic and this has been confirmed on in-house smear exam,

patient should was excluded from the study. For patient in the VPET group an extra 0.5mls were collected in EDTA for platelet count at external lab and do a blood smear.

- The selected product was prepared and administered following the protocols previously described (Chapter 2.66 for V-Pet and Chapter 2.77 for Orthokine).

Day 1, 24 hours after treatment:

- Pressure mat analysis was repeated as on day 0
- Von Frey test was repeated as on day 0
- Patient was discharged and a re-examination appointment booked for 7 days' time

Day 7

- Client was greeted and asked about progression of lameness since previous appointment, any adverse effects were noted, or medications altered (NSAIDS treatment should not have been changed). A clinical and orthopaedic examination was performed.
- Client was asked to complete re-examination LOAD questionnaire (different from questionnaire used for initial consult).
- If Orthokine patient, client was asked to sign another clinical consent form for sedation and intra-articular treatment as on Day 0.
- Pressure mat analysis was repeated as on day 0
- Von Frey test was repeated as on day 0
- V-PET patients were discharged, and appointment is booked in 3 weeks' time (4 weeks after initial treatment).
- Orthokine patients were hospitalised and the second aliquot of product prepared the previous week and stored at -18°C was administered into the previously treated elbow joint following the same protocol as before (Chapter 2.77). At discharge, an appointment was booked for 3 weeks' time (4 weeks after initial treatment).

Day 30 and Day 90

- Client was greeted and asked about progression of lameness since previous appointment, any adverse effects were noted, or medications altered (NSAIDS treatment should not have been changed). A clinical and orthopaedic examination was performed.
- Client was asked to complete re-examination LOAD questionnaire (different from questionnaire used for initial consult).
- Pressure mat analysis was repeated as on day 0
- Von Frey test was repeated as on day 0
- Patient was discharged and a re-examination booked (if day 30, for day 90).

2.9 STATISTICAL METHODS

Numerical variables were expressed as the arithmetic mean and standard deviation (±SD) and compared between groups using Students t-test for unpaired groups (V-PET vs. IRAP) or paired groups (Day 0 vs. Day 30). Statistical analysis was not possible for outcome values at day 90 due to 4 dogs failing to return for assessment at that stage. A significance level (α) was set at 0.05. Statistical analysis was performed in TIBCO Statistica 13.3 (TIBCO Software Inc., Tulsa, CA).

3 RESULTS

Note. The Covid pandemic significantly adversely affected case recruitment for this study, as the hospital was closed except to emergencies (and thereafter urgent cases) for 6-9 months at the time when the author was in the case recruitment period of the study. Lockdown also negatively impacted the author's ability to bring cases back for follow-up appointments due to limitations placed on client attendance and bringing non-urgent cases into the hospital.

Ultimately nine dogs were recruited that met the criteria for inclusion in the research project, however one dog was seen on two occasions, and has been considered as a new patient on each occasion. Four were treated with V-PET and received one intraarticular injection into the worst affected elbow. Six dogs were treated with Orthokine IRAP and therefore received two intraarticular injections, 7 days apart, into the worst affected elbow. Patients 1-4 were treated with V-PET, while patients 5-10 were treated with Orthokine. Patient '1' was initially treated with V-PET in the left elbow, and was subsequently seen again one year later, and was treated with Orthokine in the same elbow (Patient '5').

Signalment of the patients was:

Patient 1: 10-year-old Rottweiler, Female Neutered (represents later as Patient 5)
Patient 2: 11-year-old Springer Spaniel, Male Neutered
Patient 3: 10-year-old Labrador Retriever, Male Neutered
Patient 4: 5-year-old Golden Retriever, Female Neutered
Patient 5: 11-year-old Rottweiler, Female Neutered (seen previously as Patient 1)
Patient 6: 5-year-old Labrador Retriever, Male Neutered
Patient 7: 3-year-old Labrador Retriever, Male Entire
Patient 8: 9-year-old Labrador retriever, Female Neutered
Patient 9: 10-year-old Labrador retriever, Male Neutered
Patient 10: 8-year-old Springer Spaniel, Female Neutered

Four patients (patients 1,2,4 and 5) did not return for the Day 90 follow up appointment, because it was scheduled during the first COVID lockdown and had to be cancelled. Patients 6,7,8,9 and 10 did not have data acquired on Day 1 (i.e., day following the intra-articular injection) as in all these cases treatment was administered early in the morning, patients were discharged in the evening and did

not return the following day. Von Frey data are partially missing in patients 2,4 and 10, because on those occasions the test was aborted as it was perceived to be causing distress to the patient.
First and most importantly, no adverse reactions were reported to either treatment.
The results for each dog are presented in **Table 2**.

Table 2. Pressure walkway gait analysis results, LOAD scores and Von Frey values for individual dogs.

Pressure mat data: Peak Vertical Force (PVF) as a percentage of bodyweight (%bw); Vertical Impulse (VI) as a percentage of bodyweight; Symmetry Index of PVF (SI).

VonFrey value: weight in grams applied to the carpal pad with the device, which caused the dog to react. Score is the mean of five measurements.

LOAD questionnaire mobility score: scale 0-52, where a lower score represents increased mobility.

Data acquired from the forelimb that received the treatment are highlighted in **bold**.

Patient Number: 1		Treated limb: LEFT			Treatment: V-PET	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	**Left fore**	**67.8**	**-**	**-**	**60.3**	**-**
	Right fore	97.2	-	-	93.2	-
	Left hind	60.1	-	-	73.1	-
	Right hind	56.7	-	-	67.1	-
Vertical Impulse (% bodyweight)	**Left fore**	**23**	**-**	**-**	**17.2**	**-**
	Right fore	31	-	-	25.6	-
	Left hind	16.5	-	-	17.2	-
	Right hind	16.5	-	-	25.6	-
Symmetry index of PVF	Left fore / Right fore	0.7	-	-	0.65	-
	Forelimbs / Hindlimbs	1.42	-	-	1.1	-
VonFrey value (grams)	**Left fore**	**-**	**-**	**-**	**-**	**-**
	Right fore	-	-	-	-	-
LOAD score	-	38	-	-	23	-

Patient Number: 2		Treated limb: RIGHT			Treatment: V-PET	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	Left fore	69.9	70.1	63.4	70.9	-
	Right fore	**60.2**	**61.1**	**61.8**	**71.8**	**-**
	Left hind	42	43.2	43.7	46.6	-
	Right hind	51.3	48.1	45.2	42.9	-
Vertical Impulse (% bodyweight)	Left fore	14.7	16.9	12.3	12.3	-
	Right fore	**13.1**	**14.3**	**12.7**	**13.4**	**-**
	Left hind	8	9	7.6	7.5	-
	Right hind	9.9	9.8	8.3	8.3	-
Symmetry index of PVF	Right fore / Left fore	0.85	0.86	0.96	0.99	-
	Forelimbs / Hindlimbs	1.41	1.44	1.44	1.6	-
VonFrey value (grams)	Left fore	237.8	231.4	223.6	-	-
	Right fore	**196**	**155.2**	**207.6**	**-**	**-**
LOAD score	-	29	-	27	23	-

Patient Number: 3		Treated limb: LEFT			Treatment: V-PET	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	**Left fore**	**58.7**	**72.2**	**65**	**89.4**	**74.9**
	Right fore	61	70.7	72.7	84.1	76.6
	Left hind	45.4	50.3	41.4	53.7	51.3
	Right hind	42.6	46.4	38.6	45.3	46.4
Vertical Impulse (% bodyweight)	**Left fore**	**13.4**	**14.2**	**15.3**	**16.8**	**18.1**
	Right fore	14.1	14.1	18.2	16.5	19.4
	Left hind	10.1	9.2	9.4	10	11.8
	Right hind	9.7	8.5	9.4	8.7	11
Symmetry index of PVF	Left fore / Right fore	0.96	1.03	0.9	1.06	1.16
	Forelimbs / Hindlimbs	1.38	1.46	1.73	1.75	1.52
VonFrey value (grams)	**Left fore**	**397.4**	**355.6**	**290.4**	**226.6**	**334.2**
	Right fore	340.8	282.4	357	314.2	300.6
LOAD score	-	11	-	7	9	7

Patient Number: 4		Treated limb: RIGHT			Treatment: V-PET	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	Left fore	74.6	86.6	92.8	86	-
	Right fore	**71.3**	**80.1**	**79.1**	**77.8**	**-**
	Left hind	51.7	57.6	54.5	50.7	-
	Right hind	52.5	61.2	54.9	56.1	-
Vertical Impulse (% bodyweight)	Left fore	17	18.9	19.8	17.8	-
	Right fore	**16.3**	**19.1**	**18.1**	**17.4**	**-**
	Left hind	12.2	12.4	11.5	10.7	-
	Right hind	12.5	12.6	11.4	11.2	-
Symmetry index of PVF	Right fore / Left fore	0.95	0.93	0.85	0.9	-
	Forelimbs / Hindlimbs	1.41	1.4	1.57	1.54	-
VonFrey value (grams)	Left fore	400	-	368	400	-
	Right fore	**337**	**-**	**311**	**381**	**-**
LOAD score	-	13	-	12	6	-

Patient Number: 5		Treated limb: LEFT			Treatment: IRAP	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	**Left fore**	**50.4**	**50.2**	**49**	**55**	**-**
	Right fore	70.3	61.8	67.2	66.6	-
	Left hind	54.1	42.9	48.9	49.5	-
	Right hind	56.9	43.2	50.5	53.8	-
Vertical Impulse (% bodyweight)	**Left fore**	**17.3**	**18.5**	**17.7**	**19**	**-**
	Right fore	24.4	21.7	23.2	24.3	-
	Left hind	13.8	13.1	12.8	14	-
	Right hind	15.1	13.9	13.7	13.8	-
Symmetry index of PVF	Left fore / Right fore	0.72	0.82	0.73	0.84	-
	Forelimbs / Hindlimbs	1.09	1.3	1.17	1.18	-
VonFrey value (grams)	**Left fore**	**285.2**	**287**	**253**	**242**	**-**
	Right fore	376	306	364	261	-
LOAD score	-	32	-	36	18	-

Patient Number: 6		Treated limb: RIGHT			Treatment: IRAP	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	Left fore	68.3	-	67.3	71	68.6
	Right fore	**59**	**-**	**64.7**	**70.2**	**72.5**
	Left hind	39.9	-	31.5	34.3	33.7
	Right hind	38.14	-	30.2	30.3	33.3
Vertical Impulse (% bodyweight)	Left fore	21.8	-	26.4	28.8	27.1
	Right fore	**19**	**-**	**24.4**	**27.9**	**27.2**
	Left hind	19.28	-	11.6	13.9	12.7
	Right hind	11.42	-	24.4	12.5	12.3
Symmetry index of PVF	Right fore / Left fore	0.86	-	0.96	0.98	1.05
	Forelimbs / Hindlimbs	1.66	-	2.14	2.18	2.12
VonFrey value (grams)	Left fore	304	-	230	201	199
	Right fore	**280**	**-**	**264**	**265**	**265**
LOAD score	-	28	-	21	14	14

Patient Number: 7		Treated limb: LEFT			Treatment: IRAP	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	**Left fore**	**72.1**	**-**	**57**	**63.6**	**94**
	Right fore	79.7	-	74.8	67.2	96.9
	Left hind	34.3	-	29.6	30.7	44.8
	Right hind	39.5	-	35	35.6	47.7
Vertical Impulse (% bodyweight)	**Left fore**	**19.4**	**-**	**20.8**	**19.7**	**26.3**
	Right fore	23.5	-	29.1	22.6	27.9
	Left hind	9.3	-	10.7	9.5	10.6
	Right hind	10.2	-	13.1	10.7	12
Symmetry index of PVF	Left fore / Right fore	0.92	-	0.77	0.95	0.98
	Forelimbs / Hindlimbs	2.08	-	2.05	1.97	2.14
VonFrey value (grams)	**Left fore**	**172**	**-**	**225**	**169**	**190**
	Right fore	293	-	342	239	270
LOAD score	-	10	-	10	6	2

Patient Number: 8		Treated limb: LEFT			Treatment: IRAP	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	**Left fore**	**79.1**	**-**	**58.9**	**67.6**	**56.2**
	Right fore	68.1	-	59.3	60.6	67
	Left hind	68.7	-	49.2	64.1	54.7
	Right hind	74.1	-	55.5	62.9	63.4
Vertical Impulse (% bodyweight)	**Left fore**	**14.8**	**-**	**16.5**	**14.8**	**14.9**
	Right fore	12.6	-	16.1	13.5	17.8
	Left hind	10.8	-	12.7	14.8	13.2
	Right hind	11.5	-	14.9	12.7	15.4
Symmetry index of PVF	Left fore / Right fore	1.17	-	1	1.13	0.86
	Forelimbs / Hindlimbs	1.03	-	1.15	1.01	1.05
VonFrey value (grams)	**Left fore**	**186**	**-**	**364**	**293**	**146**
	Right fore	379	-	386	163	182
LOAD score	-	29	-	25	24	26

Patient Number: 9		Treated limb: RIGHT			Treatment: IRAP	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	Left fore	63.8	-	66.8	66.4	68.7
	Right fore	**63.4**	**-**	**53.3**	**56.9**	**58.5**
	Left hind	38.5	-	43.6	39.9	45.2
	Right hind	41.8	-	36.4	34.3	43.4
Vertical Impulse (% bodyweight)	Left fore	28.4	-	37.9	32.1	31.8
	Right fore	**30.5**	**-**	**32.2**	**29.4**	**30.7**
	Left hind	18.8	-	25.9	21.2	22.6
	Right hind	18.9	-	19.8	17.9	22.5
Symmetry index of PVF	Right fore / Left fore	0.99	-	0.79	0.85	0.85
	Forelimbs / Hindlimbs	1.58	-	1.5	1.66	1.44
VonFrey value (grams)	Left fore	279	-	248	256	369
	Right fore	**380**	**-**	**361**	**308**	**390**
LOAD score	-	32	-	29	27	28

Patient Number: 10		Treated limb: LEFT			Treatment: IRAP	
		Day 0	Day 1	Day 7	Day 30	Day 90
Peak Vertical force (% bodyweight)	**Left fore**	**51.1**	**-**	**46.4**	**43.2**	**49.3**
	Right fore	92.9	-	57.3	63	63.3
	Left hind	49.9	-	34.4	33	44.4
	Right hind	45.8	-	34	32.9	36.1
Vertical Impulse (% bodyweight)	**Left fore**	**9.6**	**-**	**13.3**	**13**	**13.7**
	Right fore	16.4	-	17.1	20.1	20.3
	Left hind	8.1	-	9.3	9.7	11.7
	Right hind	7.9	-	9.3	10	9.9
Symmetry index of PVF	Left fore / Right fore	0.56	-	0.82	0.7	0.72
	Forelimbs / Hindlimbs	1.51	-	1.52	1.6	1.47
VonFrey value (grams)	**Left fore**	**141**	**-**	**41.6**	**59.2**	**-**
	Right fore	192	-	139.4	98	-
LOAD score	-	21	-	20	22	21

As would be expected in most cases peak vertical forces were higher in the forelimbs compared to the hindlimbs, and lower in the lame fore leg compared to the contralateral less affected forelimb. The latter was also reflected in the symmetry indices, and VonFrey scores were also lower in the lame leg of most cases.

Following treatment, six out of 10 patients, showed increased PVF of the treated forelimb at Day 30 compared to Day 0, although the change was not statistically significant (see Tables 3 below). Interestingly, while this was also reflected in a decrease in LOAD scores, indicating the owners perceived an improvement, but this was not reflected by the VonFrey scores, except in case 4.

Table 3. Pressure mat gait analysis data, VonFrey value and LOAD scores for treatment groups, presented as mean (± SD).

Treatment	**Peak Vertical Force** (% bw) **of treated forelimb** Mean ± SD		*p-values*
	Day 0	**Day 30**	
V-PET (n=4)	64.5 ± 6.0	74.8 ± 12.1	0.282
IRAP (n=6)	59.3 ± 13.7	59.4 ± 9.9	0.974
p-values	0.499	0.058	

Treatment	**Vertical impulse** (% bw) **of treated forelimb,** Mean ± SD		*p-value*
	Day 0	**Day 30**	
V-PET (n=4)	16.5 ± 4.6	16.2 ± 1.9	0.907
IRAP (n=6)	16.8 ± 4.1	20.6 ± 6.7	0.067
p-value	0.907	0.242	

Treatment	**Peak Vertical Force** (% bw) **of UNTREATED, contralateral forelimb** Mean ± SD		*p-values*
	Day 0	**Day 30**	
V-PET (n=4)	75.6±15.4	83.5±9.3	0.281
IRAP (n=6)	73.5±10.7	65.8±3.6	0.167
p-values	0.829	0.0026	

Treatment	**Vertical impulse** (% bw) **of UNTREATED, contralateral forelimb** Mean ± SD		*p-value*
	Day 0	**Day 30**	
V-PET (n=4)	19.2±7.9	18.05±5.5	0.82
IRAP (n=6)	21.2±5.7	23.56±6.56	0.106
p-value	0.656	0.204	

Treatment	**Peak Vertical Force** (% bw) **of hindlimb ipsilateral to treated forelimb** Mean ± SD		*p-values*
	Day 0	**Day 30**	
V-PET (n=4)	52.3±6.0	56.4±12.50	0.435
IRAP (n=6)	47.8±12.6	40.3±13.6	0.345
p-values	0.529	0.095	

Treatment	**Vertical impulse** (% bw) **of hindlimb ipsilateral to treated forelimb** Mean ± SD		*p-value*
	Day 0	**Day 30**	
V-PET (n=4)	12.2±3.1	11.7±3.9	0.36
IRAP (n=6)	12.0±3.9	13.1±3.2	0.206
p-value	0.933	0.55	

Treatment	**Peak Vertical Force** (% bw) **of hindlimb contralateral to treated forelimb** Mean ± SD		*p-values*
	Day 0	**Day 30**	
V-PET (n=4)	48.3±7.2	54.42±10.05	0.616
IRAP (n=6)	49.1±14.0	43.2±12.3	0.043
p-values	0.92	0.17	

Treatment	**Vertical impulse** (% bw) **of hindlimb contralateral to treated forelimb** Mean ± SD		*p-value*
	Day 0	**Day 30**	
V-PET (n=4)	11.6±3.7	13.1±8.4	0.59
IRAP (n=6)	13.8±4.7	13.7±4.0	0.949
p-value	0.4560.	0.881	

Treatment	**Symmetry Index (PVF treated forelimb / PVF contralateral forelimb)** Mean ± SD		*p-value*
	Day 0	**Day 30**	
V-PET (n=4)	0.87 ± 0.12	0.90 ± 0.18	0.545
IRAP (n=6)	0.87 ± 0.21	0.91 ± 0.15	0.424
p-value	0.977	0.933	

Treatment	**Symmetry Index (PVF forelimbs/PVF hindlimbs)** Mean ± SD		*p-value*
	Day 0	**Day 30**	
V-PET (n=4)	1.41 ± 0.02	1.5 ± 0.28	0.573
IRAP (n=6)	1.49 ± 0.39	1.6 ± 0.45	0.276
p-value	0.674	0.697	

Treatment	**Von Frey value of treated forelimb (grams)** Mean ± SD		*p-value*
	Day 0	**Day 30**	
V-PET	310.1 ± 103.4	303.8 ± 109.2	0.661
IRAP (n=6)	240.7 ± 90.1	222.7 ± 93.8	0.548
p-value	0.331	0.343	

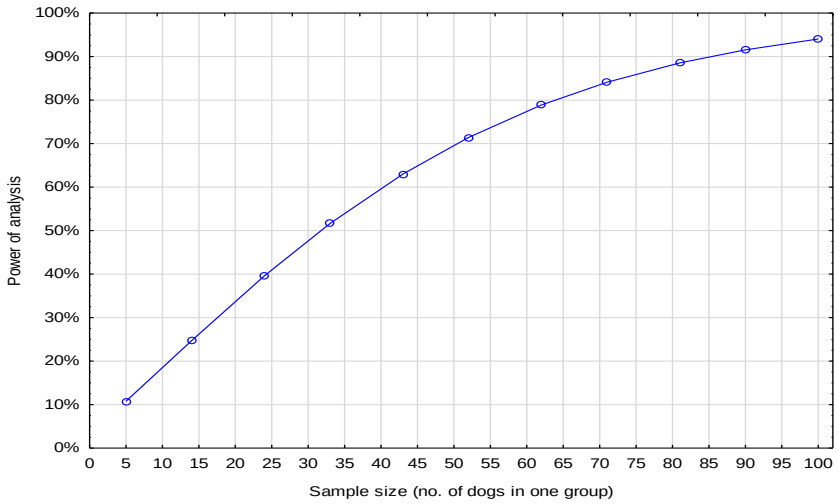
Treatment	**LOAD score** Mean ± SD		*p-value*
	Day 0	**Day 30**	
V-PET (n=4)	22.8 ± 13.0	15.3 ± 9.0	0.071
IRAP (n=6)	25.3 ± 8.5	18.5 ± 7.6	0.038
p-value	0.711	0.556	

A statistically significant improvement was noted for LOAD score at Day 30, compared to pre-treatment value (Day 0) for the Orthokine IRAP group (P=0.038). No statistically significant difference between Day 0 and Day 30 was noted for any of the other outcome measures.

Further analysis was undertaken to calculate of how many patients would be required to identify a significant difference in PVF (%bw) between the treatment groups, based on an assumption that a difference of ≥5% in PFV (%bw) would be clinically meaningful. The current sample size of 4 and 6 dogs corresponds to only 10% power, meaning we can only be 10% sure that p values > 0.05 truly indicate no significant difference between the treatment groups, or timepoints. That is, the small sample size may here predispose to a Type II error, where the null hypothesis (= no difference) is incorrectly accepted, leading to a false negative result.

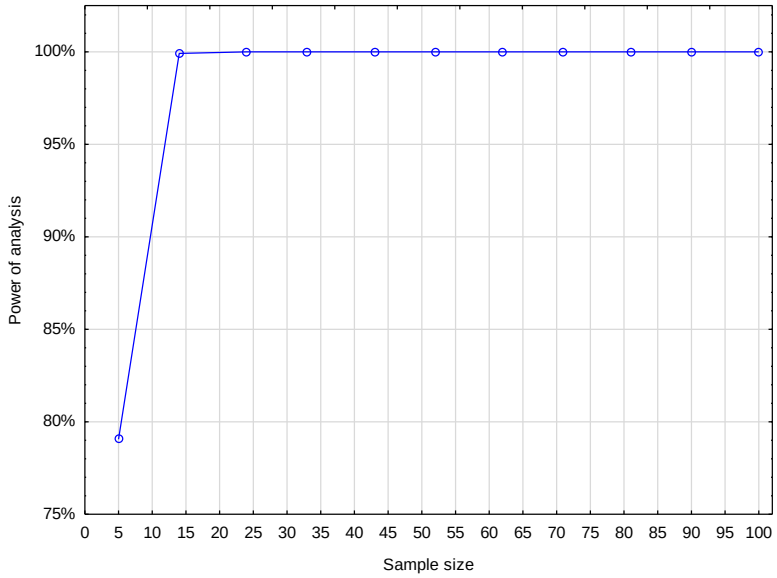
To increase the power while keeping the meaningful difference set at 5%, the sample size would need to be increased according to the graph below (Figure 44). The graph shows that acceptable power (usually considered as ≥ 80%) would be achieved by enrolling at least 60 dogs in each group.

Figure 44 Power of analysis for a difference in PVF between groups set at 5%



If the definition of clinically meaningful difference is changed from 5% to 20% then graph becomes as shown below (Figure 45). With these conditions, 10 dogs in each group would allow a power of analysis of approximately 90%.

Figure 45 Power of analysis for a difference in PVF between groups set at 20%



4 DISCUSSION

4.1 Why was this study undertaken?

4.1.1 Background

This research project started with the ambitious aim of improving the currently limited evidence on the effectiveness of two regenerative medicine modalities used for the treatment of elbow osteoarthritis in dogs. Elbow diseases are a frequent cause of lameness in both young (Demko and McLaughlin, 2005) and older dogs (Mielke *et al.*, 2018). Depending on the specific condition affecting the elbow, both surgical and non-surgical treatments can be considered to improve comfort and limb function. Irrespective of the treatment option chosen however, development of secondary osteoarthritis (OA) is unfortunately inevitable (Demko and McLaughlin, 2005; Michelsen, 2013).

Unfortunately, there is currently no effective treatment, medical or surgical, that will either cure OA, or completely resolve the clinical signs in every case (Bland, 2015). Thus, there are large numbers of patients of all species with OA, including humans, who remain chronically painful despite multimodal treatment. This presents a significant welfare concern which motivates scientists and clinicians to investigate possible treatment options for OA. The author faces this issue routinely when consulting in the orthopaedic clinic of the Small Animal Hospital, having to deal with the burden of not being able to significantly improve the quality of life of affected dogs – that are often young adult dogs with their whole life ahead of them. This is what prompted the author to investigate regenerative medicine as a potential treatment to help improve the quality of life of these patients.

4.1.2 What about elbow replacements and elbow arthrodesis?

End-stage OA affecting joints *other* than the elbow can often be managed satisfactorily with so-called 'salvage' procedures: joint replacement (as in the case of hip and knee) or arthrodesis (as in the case of tarsus, carpus and shoulder). These salvage procedures, total elbow replacement (TER) and elbow arthrodesis (EA) are possible, although neither is widely adopted. In case of TER this is likely because of the high reported complication rates, for example, 60% perioperative, 15% short-term and 15% mid-term as reported by De Sousa *et al.* (2016). For EA this is mostly because the joint cannot be fused at an angle that functions well for both standing and walking, and this causes persistent significant mechanical lameness. EA is also affected by a high complication rate with 19 of 22 dogs suffering complications reported in a recent publication (Dinwiddie *et al.*, 2021).

Finally, both elbow replacement and arthrodesis are major surgical procedures which can be performed only by a limited number of specialist surgeons and require very high financial commitment from the owner. Because of these limitations, only a very small number of patients, among those suffering from chronic elbow OA undergo elbow replacement or elbow arthrodesis. Similarly, despite the development of the Canine Unicompartmental Elbow (CUE) system, which involves resurfacing of a small portion the medial compartment of the elbow, good or acceptable functional outcomes have been reported only in one study (Cook *et al*. 2015). CUE can only be considered for patients where elbow disease is localised to the medial compartment, and it is contraindicated if diffuse elbow OA is present. To the author's knowledge, this procedure has not gained popularity due to the extensive surgical approach required to perform it, combined with the scarce evidence supporting its efficacy and safety.

4.1.3 Why explore regenerative therapies?

Regenerative medicine has evolved rapidly during recent years with new therapies based on stem cells, tissue engineering, gene therapy, and usage of autologous blood product such as platelet rich plasma (PRP) or autologous conditioned sera (ACS). In the author's opinion, in the absence of effective treatment options for OA, regenerative medicine modalities hold exciting potential. However, although the recent literature contains an overwhelming amount of information on the use of platelet therapy in human and also veterinary patients, the quality of the evidence is variable and results are conflicting. For example, Brossi et al. (2015) published a systematic review on platelet rich plasma (PRP) and included 123 studies. The authors observed that PRP's beneficial effects were reported in 46.7% of the clinical studies, while no positive effects were observed in 43.3% (Brossi *et al.*, 2015). Similarly weak evidence, and many fewer studies, exist for the use of ACS in human and veterinary patients. Despite this lack of evidence, 'regenerative medicine' is increasingly used in general veterinary practice, at significant cost to clients.

It was therefore decided to undertake this Master's project to acquire objective data to assess the potential clinical efficacy of a commonly used type of regenerative medicine (V-PET, platelet concentrate) and a less well-established but promising type (Orthokine, autologous conditioned sera).

Among the vast variety of kits available for the production of platelet concentrate the author chose to use V-PET as it is commercially available, easy to use and requires minimal additional equipment making it a viable option for routine use in clinical settings. Also, VBS direct, the retailer of the V-PET

kit in the UK, cooperated by providing the kits used for the project free of charge. In the author's experience, the kit was very easy to use, and the treatment process very efficient, as the platelet concentrate was ready for administration within an hour of the blood being collected. The steps were easy to follow, and the fact that the entire kit is sterile on delivery made it easier to avoid contamination of the final product, reducing the risk of iatrogenic septic arthritis.

Similarly, the decision to use the Orthokine system was made based on the commercial availability of the kit, and enthusiasm of the company to donate the kits and the equipment required for the study. The clinical use of the Orthokine kit in dogs has not been described in any peer review journal. The kit was easy to use, despite a greater number of steps being required, compared to the V-Pet system, to convert the patient's blood into ACS. This potentially increases the risk of contamination of the final product, but the absence of cells in the final ACS, allows the product to be filtered through a 0.22um filter prior administration, which would remove bacteria (which on average are 1um to 10um in size) and contaminants. None of the patients in this study suffered intra-articular infections following either treatment, suggesting that both are likely equally safe.

The idea of using two different treatment groups was introduced into the study design before the difficulty in recruiting cases, and indeed covid19 pandemic, became apparent and had the purpose of mitigating the lack of an ideal placebo controlled group. In hindsight, having known the low final number of cases, more meaningful data might have been obtained by allocating all patients to a single treatment group.

4.2 What were the challenges, what was learnt?

4.2.1 The pressure walkway system

A pressure sensitive walkway (PSW) was chosen for this study, due to the portability and versatility of the system, which makes it ideal for use in clinical settings. However, learning to use the system involved a very steep learning curve, complicated by the fact that - despite assurances over a period of months from the company (Tekscan) that supplied the mat, that the seemingly widely erroneous results could be explained - we were eventually able to prove to them that the system was faulty, and it had to be replaced. This caused a delay of approximately 12 months, which therefore delayed case recruitment into the trial. Testing the new system, in collaboration with Javier Rincon Alvarez and Prof. Sandra Corr, we identified that results were much more realistic, but still seemed inconsistent. In further reviewing the literature in this specific context, it was evident that although values reported

in force plate studies seemed consistent, those reported in PSW studies vary greatly (Rincon Alvarez *et al.*, 2020). Colleagues and authors of previous PSW studies were contacted, and while the significant inter-study variability was acknowledged, a consensus could not be reached on the reason(s) for it. On this basis, the focus of the Masters project of Javier Rincon Alvarez was changed completely to firstly investigating the factors that could cause such variability in PSW data, not only between studies but within a study, and then to explore this further by using the system to collect and analyse the gait of normal dogs. This generated a valuable reference study, on which the present author is a co-author, published in the main veterinary and comparative orthopaedic journal (Rincon Alvarez *et al.*, 2020).

4.2.2 Case recruitment and data collection

Based on the number of dogs with elbow disease being seen in the Small Animal Hospital, and the intention to recruit cases from referring practices, it was estimated that approximately 25 patients could be recruited over the relevant data collection period of this project. In retrospect, this was overly ambitious, overestimating the availability of suitable candidates willing to enter the trial and also the strict inclusion criteria (see paragraph 2.2) meant several potential candidates had to be excluded due to not fitting exactly these criteria. In addition, the workload required to treat and monitor the progress of each patient was significantly higher than expected.

It is the author's opinion that patient's number and workload would have been estimated more accurately if the treatment modalities investigated had been in use by the orthopaedic service before the study commenced. For future projects, the author might suggest that treatment modalities are firstly trialled by the team before being included in a prospective study. This would allow more accurate anticipation of the potential number of patients and also accurate estimate of the required time for treatment and follow up.

4.2.3 An unexpected pandemic shutting down the world

The onset of the Covid19 pandemic and consequent national lockdown caused significant disruption with both case recruitment, and follow up data collection on cases already enrolled. As previously reported, the Small Animal Hospital was running on an emergency only basis and non-urgent consultations were not allowed. It is also reasonable to assume that the general distress caused by the pandemic would negatively influence owners desire to enrol their dogs in a clinical trial, thereby limiting the recruitment of potential cases.

4.3 Results interpretation

For the majority of the dogs included in this study pressure walkway gait analysis results were in agreement with what is expected and reported in the literature.

PVF and VI were higher in the forelimbs compared to the hindlimbs, and additionally, symmetry index forelimbs/hindlimbs were always higher than 1, suggesting that all patients put more weight on their forelimbs and less on the hindlimbs, which is normal for dogs and in agreement with Voss *et al.* (2007).

Prior to treatment 8 of 10 dogs recorded, as expected, a lower PVF and VI on the most affected forelimb compared to the contralateral forelimb. This was in line with the expectation that the most affected forelimb, where intraarticular treatment was to be administered, was more painful, was loaded less, and therefore should registered lower values of PVF and VI. Similarly, lower forces in the lame leg have been reported by Horstman *et al.* (2004) and Upchurch *et al*. (2016) in dogs with cruciate disease and hip OA respectively. The only exceptions were Patient 8, who, on Day 0, recorded a higher PVF and VI in the most affected forelimb than in the contralateral, and Patient 9, who recorded a higher VI and lower PVF compared to the contralateral forelimb. These results likely reflect the fact that those two patients were almost bilaterally lame. The forelimb that was identified as being "most affected" (and therefore received treatment) based on clinical exam, owner's history and referring vet history was likely temporarily "least affected" on Day 0. It is likely that on the day of the examination, the pressure mat correctly identified that Patients 8 and 9 were putting more weight on the "most affected" limb. This hypothesis is supported by measurements at Days 7, 30 and 90 where the "most affected" forelimb was loaded less, and therefore more lame, than the contralateral. Avoiding this would have required including in the study only patients with unilateral disease and excluding patients with bilateral disease. In the majority of cases, elbow OA is secondary to developmental elbow disease, which is very often bilateral. For this reason, exclusion of patients with bilateral elbow disease would have excluded all the patients included in this study and made this research virtually impossible in our current clinical settings. Alternatively, a possibility would have been performing several measurements on different days, before the treatment is administered, to compensate for temporary shifts of the lameness. While this is theoretically possible and had been considered, it was deemed unlikely that the ethics committee would have consented to delay treatment for animals considered in chronic persistent discomfort.

VI represents the force applied on a limb over stance time and should reflect the PVF. This means that a high PVF value is usually associated with a high VI and vice versa as reported by Wustefeld-Janssens

et al. (2016). The vast majority of our data is in agreement with this statement. Examples of exceptions are Patient 6 (where from Day 0 to Day 1 an increase in VI is noted while PVF is almost static) or Patient 2 (where from Day 7 to Day 30 PVF increases while VI remains static). It is likely that these values were caused by inconsistencies in patient velocity between trials. Gait velocity affects both PVF and VI with an increase in velocity causing increase PVF and decrease in VI as reported by Hans *et al.* (2014). In this Masters project, trials were accepted if patient velocity was within specific ranges but patients were allowed to move at self-selected steady pace (See paragraph 2.3.7). This allowed for some variability in velocity and could have been prevented by forcing patients to move at a specific speed. This option was excluded during design of the study as both challenging and also likely to cause unnecessary distress to the patients.

Following administration of an effective treatment causing improvement or resolution of the lameness the following changes would be expected:

- PVF should increase in the treated leg, but it might not necessarily decrease in the other leg.
- VI may not necessarily change – as they reflect force over time. If the stance time is identical, then with an increase in peak force the impulse will increase, but if the stance time decreases, impulse may not increase.
- PVF symmetry index (SI) treated forelimb / contralateral forelimb should increase, become closer to 1. With SI forelimbs / hindlimbs, if there is overall more load put through the forelimbs, then the values should increase.
- VonFrey values should increase with treatment, indicating improvement of central pain sensitization
- LOAD scores should decrease with treatment, indicating improved owner assessed mobility.

Patient 1:

In this patient PVF and VI were lower in affected forelimb compared to the contralateral. SI left / right was less than 1, as expected, and did not change over time. SI forelimbs/hindlimbs on Day 0 was higher than 1, as expected, but decreased at Day 30, suggesting that the patient was shifting more weight onto hindlimbs after treatment. LOAD score decreased from Day 0 to Day 30 indicating an improvement of owner perceived mobility.

These data suggest that there was no improvement of gait analysis data after treatment and actually the patient was loading less weight on the treated forelimb and more on the hindlimbs at Day 30. This is in contrast with the improvement in LOAD score which indicates that the owner perceived an improvement in overall mobility. There is no VonFrey data for this patient as the device had not been delivered yet and also data for Day 1, Day 7 and Day 90 is missing as the owner could not attend the appointments. These results could either indicate that the patient overall improved after treatment, as perceived by the owner, but was temporarily not better on Day 30 when re-examined. Alternatively, they indicate the presence of "placebo effect" with a perceived improvement despite ineffective treatment.

Patient 2:

In this patient PVF prior to treatment was lower on the treated right forelimb compared to the contralateral, which is consistent with presence of right forelimb lameness and is further supported by a right/left SI which was lower than 1. PVF remained static at Day 1 and Day 7 but increasead at Day 30, while PVF values of the contralateral forelimb remained static. This is in agreement with the VonFrey values, that were lower in the right forelimb and increased following treatment and with the LOAD score which also increased. These results are all in agreement suggesting an improvement of the right forelimb lameness following treatment.

Patient 3:

For this dog, PVF prior to treatment was lower on the treated left forelimb compared to the contralateral, which is consistent with presence of left forelimb lameness and is further supported by a left/right SI which was lower than 1. PVF increased at Day 1,7, 30 and 90 while PVF values of the contralateral forelimb also increased. This suggests a persistent increase in mobility after Day 0 with more weight being put through both forelimbs. This is in agreement with the improvement in LOAD score which suggests improved perceived mobility but not with the VonFrey values which were similarly high in both forelimbs and remained almost static from day 0 to Day 90. With exception of the VonFrey values these results are in agreement suggesting improvement of lameness and mobility of this patient but raising the question if the improvement is related or not to the treatment (which

should not cause improvement in the contralateral limb). Static high VonFrey results likely indicate lack of central pain sensitization in this patient.

Patient 4:

PVF prior to treatment was lower on the treated right forelimb compared to the contralateral, which is consistent with presence of right forelimb lameness and is further supported by a right/left SI which was lower than 1. PVF increased at Day 1,7, 30 and 90 while PVF values of the contralateral forelimb also increased. This suggests a persistent increase in mobility after Day 0 with more weight being put through both forelimbs and therefore raises the question if the improvement is related to treatment (which should not cause improvement in the contralateral limb). This is in agreement with the improvement in LOAD score which suggests improved perceived mobility and with the VonFrey values which are lower in the right forelimb and improve following treatment. These results are in agreement suggesting improvement of lameness and mobility of this patient. As in Patient 3 results raise the question if the improvement is related or not to the treatment, which should not cause improvement in the contralateral limb.

Patient 5:

In this patient, PVF prior to treatment was lower on the treated left forelimb compared to the contralateral, which is consistent with presence of left forelimb lameness and is supported further by a left/right SI which was lower than 1. PVF remained static at Day 7 and was mildly increased at Day 30 while PVF values of the contralateral forelimb were mildly decreased. This suggests a mild increase in mobility at Day 30 compared to Day 0, with more weight being put through the treated left forelimb, less through the right forelimb and overall, more weight put through both forelimbs as indicated by the increased SI forelimbs/hindlimbs. With exception of the VonFrey values these results are in agreement suggesting mild improvement of lameness and mobility of this patient. Static VonFrey value results which remain lower on the treated forelimb likely indicate persistent central pain sensitization in this patient.

Patient 6

PVF prior to treatment was lower on the treated right forelimb compared to the contralateral, which is consistent with presence of right forelimb lameness and is further supported by a right/left SI which was lower than 1. PVF increased at Day 7, Day 30 and Day 90, while values of the contralateral forelimb remained static. This is in agreement with the LOAD score which increased. VonFrey values were lower on the right forelimb prior to treatment, as expected. Interestingly they remained static on the right forelimb but decreased progressively in the contralateral. With exception of the VonFrey values these results are in agreement suggesting mild improvement of lameness and mobility of this patient. VonFrey values, are difficult to interpret objectively due to the unusual trend and might indicate static central pain sensitization in the treated forelimb with worsening on the untreated forelimb.

Patient 7

PVF prior to treatment was lower on the treated left forelimb compared to the contralateral, which is consistent with the presence of left forelimb lameness and is further supported by a left/right SI which was lower than 1. Compared to Day 0, PVF of the left forelimb was lower at Day 7 and Day 30 and then significantly higher at Day 90. This suggests a worsening of lameness at Day 7 and Day 30 followed by an increase in mobility at Day 90. This is in partial agreement with the improvement in LOAD score which instead suggests progressive improved perceived mobility with no temporary worsening. VonFrey values were lower in the treated left forelimb compared to the contralateral and remained almost static. With exception of the VonFrey values these results are in agreement suggesting improvement of lameness and mobility of this patient but raise the question if the improvement is related or not to the treatment as this was only noted at Day 90, 3 months after treatment. Static VonFrey value results which remained lower on the treated forelimb likely indicate persistent central pain sensitization in this patient.

Patient 8

PVF prior to treatment was higher on the treated left forelimb compared to the contralateral, which is unusual and, as previously discussed, likely reflect bilateral nature of lameness of this patient. Compared to Day 0, PVF of the left forelimb was lower at Day 7 and Day 30 and Day 90 which suggests a worsening of lameness. This is in agreement with the VonFrey values which also decreased but not

with the LOAD score which showed a minimal improvement. With exception of the LOAD score these results are in agreement indicating worsening of mobility of this patient. The improvement in LOAD score was small and unlikely to be significant.

Patient 9

PVF prior to treatment was marginally lower on the treated right forelimb compared to the contralateral, this likely reflects the bilateral nature of lameness of this patient. Compared to Day 0, PVF of the right forelimb was marginally lower at Day 7 and Day 30 and Day 90. On the contrary PVF was marginally higher on the contralateral left forelimb which suggests a mild worsening of the right forelimb lameness with weight being shifted to the left. This is in agreement with the VonFrey values which also decreased at Day 7 and Day 30 and then increased again at Day 90, but not with the LOAD score which instead showed a minimal improvement. With exception of the LOAD score these results are in agreement indicating mild worsening of mobility of this patient. The improvement in LOAD score is small and unlikely to be significant.

Patient 10

PVF prior to treatment was lower on the treated left forelimb compared to the contralateral, which is consistent with presence of left forelimb lameness and is further supported by a left/right SI which was lower than 1. Compared to Day 0, PVF of the left forelimb remained almost static at Day 7, Day 30 and Day 90. This suggests a static nature of lameness which is in agreement with the static LOAD score. VonFrey values were lower in the treated left forelimb compared to the contralateral at Day 0 and then dropped significantly. With exception of the VonFrey values these results are in agreement suggesting persistent static left forelimb lameness in this dog. The significant drop in VonFrey values should be interpreted with caution in this patient as this was a stressed dog, who did not tolerate well manipulation of the paws, and in fact VonFrey values were not taken at Day90 for this reason. It is likely that stress and poor tolerance to manipulation might have therefore influenced the VonFrey values.

4.4 Future work

Despite the absence of statistically significant results, this study established a protocol for use of V-Pet and Orthokine, established a protocol for assessment of patient outcomes following treatment, and allowed performance of a power calculation. Patient recruitment and treatment is ongoing and will continue after submission of this thesis, which could lead to further publications.

Early on in this study, the author and colleagues developed a collaboration with the Orthopaedic team at the Queen's Veterinary School Hospital at the University of Cambridge, who began treating patients with V-PET following an identical protocol. Due to similar issues with case recruitment at Cambridge, pooling the data is currently being considered.

Another important direction for the research is to further investigate aspects of 'quality control' of both products. For example, assessment of the platelet concentration of the V-Pet product prior to injection would be relatively easy to do in the clinical environment. For both PRP and ACS treatments, investigation of the synovial fluid composition following treatment would be extremely interesting – for example, whether the type and concentration of growth factors was altered.

4.5 Limitations

There are several limitations to this study that must be acknowledged. These include the small size of the treatment groups, and the variability of the product being delivered.

"Placebo effect" is the beneficial effect produced by a placebo drug or treatment, which cannot be attributed to the properties of the placebo itself, and must therefore be due to the patient's belief in that treatment. Counterintuitively, this has been shown to be significant also in veterinary medicine, despite the fact that patients are not aware of the fact they are receiving a treatment (Zhang and Patterson, 2010). This effect is likely to be present in every study, including this research project, making interpretation of subjective outcome scores results less reliable. If "placebo effect" is present an improvement in subjective outcome score might be seen, even if the treatment was ineffective, due to the owner "perceiving" that their pet has improved, even if that was not the case. A placebo control group would allow to identify presence of placebo effect and the lack of a placebo control group represents another significant limitation of this research project. Ideally a control placebo group would have received an intraarticular injection e.g. of saline solution, or alternatively, sampling, sedation and joint puncture without administration of anything, and with the owner and assessors

blinded to the treatment group. However, the inclusion of such placebo groups was considered unethical by the author, and it is unlikely that such a study would have received ethical approval from the School Research Ethics Committee. Further, such 'placebo' treatment would have taken the study outside of normal veterinary practice, and may therefore have require Home Office approval.

In contrast to the pressure mat, which is increasingly used in clinical gait analysis, the use of the VonFrey apparatus is less well reported. Von Frey data are not commonly used outcome measure in orthopaedics but were acquired as they might help to show if treatments explored had an effect on central pain sensitization. In veterinary settings a Von Frey anaesthesiometer was used successfully to identify presence of central sensitization in dogs in association with cruciate ligament rupture (Brydges et al., 2012), hind limb OA (Williams et al., 2014; Knazovicky et al., 2016) and neuropathic pain (Kerns et al., 2019) and also in cats with hindlimb OA (Addison and Clements, 2017). There are currently no published studies demonstrating that an electronic VonFrey device is effective in identifying presence of central sensitization in dogs in association with elbow osteoarthritis. It is therefore difficult to evaluate whether the lack of statistically significant improvement in VonFrey scores post treatment is due to lack of response to treatment or, alternatively, to the fact that VonFrey is not effective in identifying presence of central sensitization in dogs with elbow osteoarthritis. The author is currently involved in an ongoing research project aimed to use a VonFrey to assess central sensitization in dogs with elbow OA.

Bias happens when a systematic error is introduced by people involved in the stidy, into the sampling or the testing by to encourage one outcome or answer over others. This can happen involuntarily and, to prevent it, patients should be randomly allocated to a treatment modality, the assessor should be blinded of which modality the patient has been assigned to and objective outcome measures should be used.

The value of subjective outcome data (i.e. validated questionnaire) is questionable as it can be affected by bias. (Muller et al., 2016b). Despite these limitations, validated questionnaires are widely used in veterinary literature and in clinical settings because assessing client perception and satisfaction is important. For this reason, the authors opted to measure client assessed outcome using a validated questionnaire (i.e., LOAD questionnaire).

Randomization was not complete in this study and the author was not blinded of the treatment modality administered to the patient and these are two significant limitations of this study. Despite this, it is unlikely that these limitations affected the final results of Pressure Mat and VonFrey as these produce objective outcomes, which the operator would not be able to influence, even if they wanted to. On the contrary, as discussed and acknowledged above, a degree of bias is possible for the LOAD questionnaire results.

4.6 Conclusions

The initial part of this project (in collaboration with JRA), showed that although the different types of calibration protocols for the pressure sensitive walkway yielded different peak vertical force and impulse values, the results were highly repeatable and reproducible for the individual protocol. In addition, the results of both protocols were strongly linearly correlated, potentially facilitating comparisons between different studies.

In the clinical stage, results did not show a statistically significant improvement in outcome measures in dogs with elbow osteoarthritis following treatment with V-PET or Orthokine. In addition, there was no statistically significant difference in the results of dogs treated with V-PET compared to Orthokine. No adverse reactions were identified following either treatment. The low number of cases in the study is likely to be contributing, at least in part, to the lack of statistical significance, and – in the absence of any adverse reactions – we intend to continue further with recruitment and treatment of patients.

With regards of the V-PET system our results are difficult to objectively compare to the available literature because, although a significant effect was not identified, only four patients were treated with V-PET making comparison of the results difficult.

With regards of the Orthokine system our results are novel as there are no published clinical trials on use of Orthokine in dogs and using objective outcome measures. The only available evidence describing clinical use of Orthokine is a non-published abstract describing use of Orthokine in 11 dogs and reporting persistent improvement in subjective lameness score, in all patients up to three months after treatment (Hauri, 2010).

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6 AUTHOR'S DECLARATION

I, Simone Anesi, declare that the work in this thesis is original, and was carried out solely by myself or with due acknowledgements. It has not been submitted in any form for another degree or professional qualification. Replication of images or figures from the authors previous publications has been done with approval of copyright from the relevant sources.

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8 APPENDICES:

8.1 Appendix 1

Small Animal Hospital
School of Veterinary Medicine
University of Glasgow
Garscube Campus
Bearsden Road
Bearsden
Glasgow
G61 1QH

Dear Pet Owner,

Regenerative Medicine for Treatment of Elbow Disease.

Thank you for considering enrolling your dog in this study. Participation is entirely voluntary and declining to be involved in no way affects the standard of care your pet will receive. If you do enrol your dog, you may change your mind and withdraw from the study at any stage. No information that identifies you will be published, in accordance with the Data Protection Act 2018.

Elbow disease is a common cause of lameness in dogs, which can be difficult to manage effectively. We are undertaking a clinical study to learn more about how affected dogs walk, and to determine whether some new commercially available treatments are effective. The study has been given ethical approval by the School Research Committee.

What would be involved? Your dog will be randomly allocated to receive one of two possible intra-articular treatments, which are given via an injection into the elbow joint. Both treatments are produced from a blood sample taken from your dog, but depending on which treatment group your pet is allocated to, the blood will be processed differently (following the kit manufacturer instructions). Both treatments aim to isolate, from the patient's own blood, molecules or cells that can reduce pain and inflammation associated with arthritis. Because the treatments are derived from the animal's own blood, the possibility of adverse allergic or anaphylactic side effects is drastically reduced.

What will it cost? There are no additional costs as a result of your dog being enrolled in the study. You will incur the cost associated with administration of the treatment (e.g. blood test, sedation, hospitalization, etc), which is approximately £350. However, the actual product will be given free of charge, as will the additional consultations and assessments (at 1 week, 4 weeks, 3 months and 6 months).

The only difference that being in the study will make to the treatment your pet would otherwise receive is that their lameness will be assessed using a pressure mat, and a Von Frey tool. The pressure mat records how he/she is distributing their weight across their paws as they walk – the mat is almost flush with the floor. The Von Frey tool is a small plastic filament placed on to the pad of the paw to see how sensitive the dog is to light pressure – as demonstrated. Dogs with arthritis have been found to have increased sensitivity and tend to react earlier to pressure on their paw. We are interested to see if the joint injections reduce the sensitivity created by the arthritis. Both the pressure mat and Von Frey tests are easily avoidable, meaning your pet can walk off the mat or away from the filaments at any time. There will also be additional re-examinations required at 7 days, 4 weeks, 3 months and 6 months.

What are the potential risks? There are no specific risks associated with the pressure mat or von Frey tool. There are minimal risks associated with the treatment itself - these include risk of joint infection, which are possible with any intra-articular injection. All the procedures will be performed with strict sterile technique to minimise this risk. Also, a degree of risk is associated sedation or general anaesthetic. A clinical examination will be performed before any sedation or anaesthetic is administered and you pet will be carefully monitored throughout to minimise the risk of any complications.

Your pet will be allocated one of the following treatments:

Treatment 1: Platelet concentrate therapy, V-PET®, Pall. A blood sample is obtained from the patient (usually from a neck vein). The blood is then passed through a specific filter system that isolates platelets from other cells. The platelets are retrieved from the filter in a sterile manner and then injected into the patient's affected elbow. Platelets are rich in molecules that can help control inflammation and pain, and also promote healing of damaged tissues. Patients allocated to this group will receive one intra-articular treatment.

Further information can be found on the manufacturer's website. *https://medical.pall.com/en/veterinary-platelet-enhancement-therapy---innovative-treatment-f.html*

<u>Treatment 2</u>: Autologous conditioned sera (Orthokine® vet, Orthogen). A blood sample is obtained from the patient (usually from neck vein). The blood is stored in a tube which contains specifically designed glass beads, and is left to incubate for 6-9 hours. During this time monocytes (a type of white blood cell) react with the glass beads and release regenerative and anti-inflammatory molecules. The tube is then centrifuged and the serum containing the proteins is retrieved and injected into the patient's affected elbow joint. Patients allocated to this group will receive two intra-articular treatments one week apart.

Further information can be found on the manufacturer' website: *https://orthogen.com/irap/en/products/orthokine-vet-irap/*

These treatments are both commercially available for routine veterinary use in the UK.

If you have any questions about any aspect of this study or are interested in enrolling your pet, please do not hesitate to contact us via the email addresses below.

Thank you for your time.

Yours faithfully,

Dr Simone Anesi: *s.anesi.1@research.gla.ac.uk*

Professor Sandra Corr: *sandra.corr@glasgow.ac.uk*

8.2 Appendix 2

REGENERATIVE MEDICINE STUDY, EXAMINATION SHEET

DATE:

VETERINARY SURGEON:

PATIENT NAME: OWNER SURNAME:

SIGNALMENT

Breed: Age: Sex:

Weight: BCS: /9

CLINICAL HISTORY

Forelimb affected (or most affected in bilateral cases): LEFT RIGHT

DIAGNOSIS:

Lameness started (date):

IMAGING PERFORMED: RX CT DATE:

Progression: STATIC IMPROVING WORSENING

Previous medical treatment and effect:

Current medical treatment and effect:

Arthroscopic treatment: YES NO DATE:

Recent blood work performed: YES NO DATE:

Blood work abnormalities:

CLINICAL EXAMINATION

ORHTOPAEDIC EXAMINATION, (To be checked by a second observer, NAME: _____)

Gait examination:

Lameness: LF RF LH RH Grade: /10

ROM: Right elbow: Left elbow:

Discomfort level (none, mild, moderate, severe): Right elbow: Left elbow:

If multiple abnormal joints, is the elbow likely the main cause of the lameness? Yes No

Notes:

Neurological abnormalities:

8.3 Appendix 3

Consent form

Effects on intra-articular regenerative therapy in dogs with elbow osteoarthritis

I agree that my pet ……..can undergo the following tests as part of this scientific study:

- Physical examination by two different veterinarians – including a general physical exam, orthopaedic examination and neurological examination
- Assessment of light touch threshold using the Von Frey monofilaments
- Assessment of gait analysis by walking across a pressure platform

Print Name: ……

Owner Signature …… Date……..

I agree that my pet ……. can receive either intra-articular treatment discussed above injected into their affected elbow joint for treatment for osteoarthritis under sedation/general anaesthesia.

Print Name:……..

Owner Signature …… Date……..

8.4 Appendix 4

REGENERATIVE MEDICINE STUDY, VON FREY TEST

DATE:

VETERINARY SURGEON:

PATIENT NAME: OWNER SURNAME:

TREATMENT GROUP: V-PET ORTHOKINE

TREATMENT DATE:

AFFECTED ELBOW: LEFT RIGHT

- Perform test in a quiet environment. Before start testing give patient 10 minutes off the lead to relax in the room (this time can be used to prepare and calibrate the device)
- One assistant gently restrains the patient while the operator performs the test. The VonFrey screen should be visible only to the assistant and the operator should be blinded of the value.
- With the patient standing the operator points the tip of the device on the middle of the carpal pad. Apply a progressive force to the device until the patient reacts (withdrawal of the paw, escape movement, vocalization) OR 400g of pressure is reached. As the operator should not be allowed to see the measured value, the assistant should say if the limit of 400g of pressure is reached. Withdrawal of the paw at first light contact with the von Frey is not considered a valid trial.
- Perform 5 valid reading in each carpal pad. Left and right measurement should be alternated (so that if patient reaction changes with time or with patient getting used to the test this will not bias the results)

	LEFT FORELIMB
1	g
2	g
3	g
4	g
5	g

RIGHT FORELIMB
g
g
g
g
g

8.5 Appendix 5

UNIVERSITY OF LIVERPOOL

Initial Visit

Liverpool Osteoarthritis in Dogs (LOAD)

Owner questionnaire for dogs with mobility problems

Dear Owner,

Thank you for agreeing to complete this questionnaire.

Your assistance in this endeavour will enable us to gather valuable information about your pet, and is a vital component in our ongoing quest to combat painful and debilitating diseases such as arthritis. It is important that all questions are answered to the best of your ability and if you have a question regarding the questionnaire, please contact a member of staff from your veterinary clinic.

Thank you again for your help.

Answering the questions

Most of the questions are fairly simple. It is important that you only tick one box per question except where otherwise requested (e.g. Question 4 under Lifestyle).

If you are in any doubt as to how to answer a particular question, please contact a member of staff for assistance.

Owner's name: Pet's name:

Owner's phone number: Client number: Today's date: YYYY-MM-DD

Breed of pet: Pet's age: Sex: M F

For office use only Reference limb: LF RF LH RH Reset

Background

1. How long has your pet been suffering with his/her mobility problem?

Up to 6 months | 6–12 months | 12–24 months | 24–36 months | more than 36 months

2. Has your dog been diagnosed as suffering from any other problems in addition to his/her orthopaedic disease?

No | Yes

Please list these if you can:

3. If you can, please list any medications that your pet is currently receiving, stating when he/she received the last dose of each:

Lifestyle

1. In the last week, on average, how far has your dog exercised each day?

- 0–1 km (0–0.6 miles)
- 1–2 km (0.6–1.2 miles)
- 2–3 km (1.2–1.9 miles)
- 3–4 km (1.9–2.5 miles)
- more than 4 km (more than 2.5 miles)

2. In the last week, on average, how many walks has your dog had each day?

- 0
- 1
- 2
- 3
- 4
- more than 4

3. What type of exercise is this?

- Always on lead
- Mostly on lead
- Mostly off lead
- Always off lead
- Working

4. Are there particular days of the week upon which your dog has significantly more exercise?
(Tick more than one box if necessary.)

- Monday
- Tuesday
- Wednesday
- Thursday
- Friday
- Saturday
- Sunday

Reset

5. On what sort of terrain does your dog most often exercise?

- On level grass
- In woodland
- On street
- Over rough hill ground

6. At exercise, how is your dog handled?

- Walk on lead
- Walk off lead
- Trot
- Run freely

7. Who limits the extent to which your dog exercises?

- You
- Your dog

Initial Visit

Mobility

For office use only

Generally

1. How is your dog's mobility in general?

○ Very good ○ Good ○ Fair ○ Poor ○ Very poor

2. How disabled is your dog by his/her lameness?

○ Not at all disabled ○ Slightly disabled ○ Moderately disabled ○ Severely disabled ○ Extremely disabled

3. How active is your dog?

○ Extremely active ○ Very active ○ Moderately active ○ Slightly active ○ Not at all active

4. What is the effect of cold, damp weather on your dog's lameness?

○ No effect ○ Mild effect ○ Moderate effect ○ Severe effect ○ Extreme effect

5. To what degree does your dog show stiffness in the affected leg after a 'lie down'?

○ No stiffness ○ Mild stiffness ○ Moderate stiffness ○ Severe stiffness ○ Extreme stiffness

At exercise

6. At exercise, how active is your dog?

○ Extremely active ○ Very active ○ Fairly active ○ Not very active ○ Not at all active

7. How keen to exercise is your dog?

○ Extremely keen ○ Very keen ○ Fairly keen ○ Not very keen ○ Not at all keen

8. How would you rate your dog's ability to exercise?

○ Very good ○ Good ○ Fair ○ Poor ○ Very poor

For office use only

9. What overall effect does exercise have on your dog's lameness?

○ No effect ○ Mild effect ○ Moderate effect ○ Severe effect ○ Extreme effect

10. How often does your dog rest (stop/sit down) during exercise?

○ Never ○ Hardly ever ○ Occasionally ○ Frequently ○ Very frequently

11. What is the effect of cold, damp weather on your pet's ability to exercise?

○ No effect ○ Mild effect ○ Moderate effect ○ Severe effect ○ Extreme effect

12. To what degree does your dog show stiffness in the affected leg after a 'lie down' following exercise?

○ No stiffness ○ Mild stiffness ○ Moderate stiffness ○ Severe stiffness ○ Extreme stiffness

13. What is the effect of your dog's lameness on his/her ability to exercise?

○ No effect ○ Mild effect ○ Moderate effect ○ Severe effect ○ Extreme effect

Thank you once again for completing this questionnaire.
Please return the form to a member of staff.

For office use only

Clicking LOAD Score will tabulate your score once.
Reset is not available for this function.

LOAD Score =

NOVARTIS ANIMAL HEALTH

UNIVERSITY OF LIVERPOOL

Follow-up Visit

Liverpool Osteoarthritis in Dogs (LOAD)

Owner follow-up questionnaire for dogs with mobility problems

Dear Owner,

Thank you for agreeing to complete this follow-up questionnaire.

Your assistance in this endeavour will enable us to gather valuable information about your pet, and is a vital component in our ongoing quest to combat painful and debilitating diseases such as arthritis. It is important that all questions are answered to the best of your ability and if you have a question regarding the questionnaire, please contact a member of staff from your veterinary clinic.

Thank you again for your help.

Answering the questions

Most of the questions are fairly simple. It is important that you only tick one box per question.

If you are in any doubt as to how to answer a particular question, please contact a member of staff for assistance.

Owner's name: Pet's name:

Owner's phone number: Client number: Today's date: YYYY-MM-DD

Breed of pet: Pet's age: Sex: M F

For office use only Reference limb: LF RF LH RH Reset

Mobility

For office use only

Generally

1. How is your dog's mobility in general?

○ Very good ○ Good ○ Fair ○ Poor ○ Very poor

2. How disabled is your dog by his/her lameness?

○ Not at all disabled ○ Slightly disabled ○ Moderately disabled ○ Severely disabled ○ Extremely disabled

3. How active is your dog?

○ Extremely active ○ Very active ○ Moderately active ○ Slightly active ○ Not at all active

4. What is the effect of cold, damp weather on your dog's lameness?

○ No effect ○ Mild effect ○ Moderate effect ○ Severe effect ○ Extreme effect

5. To what degree does your dog show stiffness in the affected leg after a 'lie down'?

○ No stiffness ○ Mild stiffness ○ Moderate stiffness ○ Severe stiffness ○ Extreme stiffness

At exercise

6. At exercise, how active is your dog?

○ Extremely active ○ Very active ○ Fairly active ○ Not very active ○ Not at all active

7. How keen to exercise is your dog?

○ Extremely keen ○ Very keen ○ Fairly keen ○ Not very keen ○ Not at all keen

8. How would you rate your dog's ability to exercise?

○ Very good ○ Good ○ Fair ○ Poor ○ Very poor

Follow-up Visit

For office use only

9. What overall effect does exercise have on your dog's lameness?

○ No effect ○ Mild effect ○ Moderate effect ○ Severe effect ○ Extreme effect

10. How often does your dog rest (stop/sit down) during exercise?

○ Never ○ Hardly ever ○ Occasionally ○ Frequently ○ Very frequently

11. What is the effect of cold, damp weather on your pet's ability to exercise?

○ No effect ○ Mild effect ○ Moderate effect ○ Severe effect ○ Extreme effect

12. To what degree does your dog show stiffness in the affected leg after a 'lie down' following exercise?

○ No stiffness ○ Mild stiffness ○ Moderate stiffness ○ Severe stiffness ○ Extreme stiffness

13. What is the effect of your dog's lameness on his/her ability to exercise?

○ No effect ○ Mild effect ○ Moderate effect ○ Severe effect ○ Extreme effect

Thank you once again for completing this questionnaire.
Please return the form to a member of staff.

For office use only

Clicking LOAD Score will tabulate your score once.
Reset is not available for this function.

LOAD Score =

NOVARTIS ANIMAL HEALTH

8.6 Appendix 6

University of Glasgow | School of Veterinary Medicine

06 November 2018

Simone Anesi
Small Animal Hospital
University of Glasgow
Glasgow
G61 1BD

Dear Simone,

Clinical Research involving animal subjects, material or data

The Research Ethics Committee has approved your application entitled "Effects of intra-articular regenerative therapy in dogs with elbow osteoarthritis." (Ref 40a/18).

Please find enclosed a signed copy for your records.

Yours sincerely,

Professor Joanna Morris
Convenor
Research Ethics Committee

School of Veterinary Medicine
464 Bearsden Road
Glasgow G61 1QH
The University of Glasgow, charity number SC004401

8.7 Appendix 7

Veterinary Medicines Directorate

Veterinary Medicines Directorate
Woodham Lane, New Haw
Addlestone, Surrey
KT15 3LS
United Kingdom

Tel: +44 (0)1932 336911
Search for VMD on GOV.UK

THE VETERINARY MEDICINES REGULATIONS 2013 SI 2013/2033

ANIMAL TEST CERTIFICATE No: **ATC-S-114**

In the name of V-PET - platelet rich product and ORTHOKINE – autologous conditioned sera containing interleukin receptor antagonist protein

University of Glasgow
464 Bearsden Road
Glasgow
G61 1QH

In accordance with the Veterinary Medicines Regulations I approve the administration of **V-PET - platelet rich product and ORTHOKINE – autologous conditioned sera containing interleukin receptor antagonist protein** to animals in accordance with the documentation submitted to the Veterinary Medicines Directorate on 21 January 2019.

This certificate is valid for two years.

Application No: 01500/2018

Signature:

A person authorised to sign on behalf of the Secretary of State for Environment, Food and Rural Affairs.

Date: 26 February 2019

Requirements to be complied with by the ATC Holder

1. The holder of this Animal Test Certificate must comply with the duties of the UK's Animal Test Certificate (ATC) scheme as explained on GOV.UK.

2. The holder must also comply with the duties, as to pharmacovigilance, set out in the Veterinary Medicines Regulations.

The holder's attention is also drawn to the following:

3. An ATC may be revoked if:

    - The ATC holder fails to observe any of the terms and conditions of the ATC;
    - Doubts arise about the safety or quality of the product;
    - Changes in the conduct of the test have an adverse effect on the safety of target or other animals, of consumers of the produce of target animals, of users of the product or of the environment;
    - Information supplied at the time of application is found to have been deficient or incorrect in a material way (i.e. in a way which influenced the VMD's decision).

ISSUED SUBJECT TO THE FOLLOWING CONDITIONS:

1. The applicant has committed to contact the VMD and justify any increase in animal numbers above those already stated (25 per group) resulting from a power calculation performed after pilot data have been acquired.
2. The applicant should add the agreed user safety warning to the product information kept with the samples for reference as required.