

KNOTLESS BIDIRECTIONAL BARBED VERSUS SMOOTH POLYPROPYLENE THREE-LOOP PULLEY  
SUTURES FOR REPAIR OF CANINE GASTROCNEMIUS TENDON

BY

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THESIS

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## ABSTRACT

Canine common calcaneal (Achilles) tendon injury is a well-known cause of lameness in the dog. The Achilles tendon complex is primarily composed of the gastrocnemius tendon, with smaller contributions from the superficial digital flexor tendon and the common tendon of the biceps femoris, semitendinosus and gracilis muscles. The primary goal in tendon repair is to provide strength appropriate to allow healing while minimizing gap formation. A large gap (>3 mm) in repaired tendon results in slower healing and decreased ultimate tensile strength (UTS) at 6 weeks, compared to those with a smaller gap.

Barbed sutures are a relatively recent entry into the veterinary field. These sutures have found multiple applications in human medicine, including digital flexor tendon repair, as well as gastrointestinal, gynecologic, urologic and cosmetic surgeries. Recent veterinary studies demonstrate that knotless barbed sutures have supraphysiologic, and in some cases, increased load to failure compared to conventional sutures in selected soft tissue surgeries. Each barb provides a small component of the total anchoring force which cumulatively can exceed the tensile strength of the smooth monofilament sutures. The barbs increase suture-tendon interface and theoretically distribute load more evenly throughout the construct, as well as minimize the chances of suture pull-out as a mode of failure. As these sutures are processed from existing materials and result in a smaller core diameter, it is recommended to select a barbed suture 1-2 sizes larger than the appropriate smooth suture, with the expectation of at least equal tensile strength. We hypothesized that a knotless barbed polypropylene repair would have increased strength compared to a smooth knotted polypropylene repair when

placed in the clinically utilized three-loop pulley (3LP) suture pattern in an in-vitro model for gastrocnemius tendon repair.

Common calcaneal tendons and calcanei were obtained as paired units from pelvic limbs of adult, sexually intact mixed breed dogs euthanized for unrelated reasons and free of gross evidence of orthopedic disease. Extraneous tissues were removed, leaving only the gastrocnemius tendon. Each bone-tendon unit was wrapped in towels moistened with saline (0.9% NaCl) solution and stored at -20°C until testing. The units were thawed to room temperature overnight prior to testing.

Tensile testing was performed with a servohydraulic testing machine equipped with a 2 kN load cell. Constructs were positioned in the testing machine at a 135° angle to mimic physiological load direction. Tendons were preloaded to 2 N, allowing precise transection 3 cm proximal to the tuber calcanei. One of each paired unit was randomly selected to receive a 3LP repair with 2-0 USP smooth monofilament polypropylene suture while the other received 3LP repair with 1 USP bi-directional barbed monofilament polypropylene suture. Ultimate tensile strength, load to 1 mm and 3 mm gap, and mode of failure were recorded with a material testing system synchronized with digital video. This data was analyzed for normal distribution and compared with Student's T-tests. If data was non-normally distributed, Mann-Whitney U tests were performed. Significance was set at  $P \leq 0.05$  for all tests.

Thirty-three paired limbs were obtained from dogs weighing between 15.9-27.2 kg (average 21.8 kg, range 16.8-27 kg). Significantly higher ultimate tensile strength ( $p < 0.001$ ) for smooth ( $40.5 \pm 10.6$  N) compared to the barbed suture repairs ( $28.6 \pm 7.1$  N) was noted. The

1mm gap load for the barbed repair ( $17.2 \pm 5.3$  N) was significantly less ( $p < 0.001$ ) than the smooth repair ( $26.8 \pm 7.1$  N). The 3mm gap load for the barbed ( $25.3 \pm 5.7$  N) was also significantly lower ( $p < 0.001$ ) than the smooth repair ( $35.1 \pm 9.4$  N).

Thirty-one (94%) out of thirty-three barbed sutures failed when the barbs reversed direction under tensile loading and the suture backed out from its initial placement. Two (6%) barbed sutures broke under tension during testing. With regards to the smooth polypropylene, 31/33 constructs failed by suture material pulling through the tendon. In these cases, the knot and suture material remained intact. The remaining two constructs failed by tendon tears proximal to the repair.

The results demonstrate that the load results at each individual sample point (1 mm gap, 3 mm gap, and UTS) were significantly lower for the barbed than the smooth polypropylene repairs. The p-values for each normally distributed data set were actually an order of magnitude beyond the threshold of significance established prior to testing with our power analysis ( $P < 0.001$ ). Based on our results, knotless barbed polypropylene repair is not a suitable alternative to smooth knotted polypropylene repair when placed in 3LP pattern for canine gastrocnemius repair *in vitro*.

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## CHAPTER 1

### INTRODUCTION

Rupture of the common calcaneal tendon (Achilles) is the second most frequent tendon rupture in dogs after the proximal biceps tendon (Jopp and Reese 2009). Presentation can range from acute transections and lacerations to complete ruptures (Lister, Renberg et al. 2009). Damage to the Achilles can result in plantigrade stance of the limb in question and significant lameness (Nielsen and Pluhar 2006; Lister, Renberg et al. 2009). Achilles injuries are generally classified as acute or chronic, and then further subdivided based on the degree of injury. The injury may be partial, where one or more component remains intact, or complete. Partial rupture is common in the veterinary literature (Rivers, Walter et al. 1997; Carmichael and Marshall 2012). Regardless of whether the rupture is partial or complete, surgical repair is the current recommendation to offer the best chance at restoration of limb function (Carmichael and Marshall 2012; Perry, Harper et al. 2014)<sup>1</sup>.

The canine Achilles is comprised of three main components: the superficial digital flexor tendon, the gastrocnemius tendon, and the combined tendon of the gracilis, biceps femoris, and semitendinosus muscles. The gastrocnemius tendon forms the most substantial and powerful portion of the Achilles, but also has the lowest load to failure (Jopp and Reese 2009; Corr, Draffan et al. 2010; Gilbert, Shmon et al. 2010). It is thought that the relatively avascular

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<sup>1</sup> This chapter contains previously published material from: Perry, B. S., T. A. Harper, et al. (2014). "Barbed versus smooth polypropylene three-loop pulley sutures for repair of canine gastrocnemius tendon." *Vet Comp Orthop Traumatol* **27**(6): 436-440. Reproduced here for educational/teaching purposes only with permission of the publisher.

fibrocartilaginous region of the distal gastrocnemius tendon may predispose that anatomic location to acute injury (Corr, Draffan et al. 2010; Gilbert, Shmon et al. 2010).

Underlying causes for canine Achilles rupture are poorly understood. Conversely, in people, many factors play a role in the development of Achilles tendonopathy. These factors include the anatomic variations in the underlying vascular supply, overstress or overuse, and biomechanical issues such as increased repetitive strain (Maffulli and Kader 2002; Paavola, Kannus et al. 2002; Jarvinen, Kannus et al. 2005). Once repaired, there is an additional preference in human medicine to minimize joint immobilization and return to controlled range of motion with early rehabilitation. This has been shown to increase repair strength in the early healing phase, as well as lessen healing time and improve joint health by minimizing immobilization of the ankle (Murrell, Lilly et al. 1994; Pneumatics, McGarvey et al. 2000; Benthien, Aronow et al. 2006).

While early mobilization and controlled rehabilitation has been demonstrated to help in the healing process in human Achilles injuries, it is a difficult proposition in animals due to the need for human intervention to ensure compliance. Therefore, to allow some return to weight-bearing activities, the repair performed on canine patients must be able to withstand an increased amount of tensile loading relative to the human equivalent. Coaptation, whether external or internal, is a more challenging proposition in canine patients due to their inability to understand the need for these types of supportive care. To address the need for controlled weight-bearing, a variety of options have been attempted in the clinical setting, however, the



goal remains to continually improve the strength of the primary repair through alternative materials, techniques, and adjunctive therapies.

Alternative repair concepts have been attempted in human medicine, often using canine models in their studies. One of the most documented adjunctive therapies, porcine small intestinal submucosa has been utilized as an additional component to sutured repair in canine primary Achilles repair. These grafts provide matrices for cellular ingrowth and show organization and constituent components comparable to normal tissues by 90 days post-operatively (Gilbert, Stewart Akers et al. 2007). Additionally, these submucosa-enhanced tendon repairs are stronger than those repaired primarily without submucosa at 12 weeks post-operatively (Badylak, Tullius et al. 1995). A polypropylene mesh was also tested both alone and in combination with a 3LP pattern for primary Achilles injury, and as a combination was noted to have a higher ultimate tensile strength. However, there was no significant decrease in gap formation with the combination of mesh and suture (Gall, Santoni et al. 2009). Other repair techniques described include both polyblend sutures with suture buttons, barbed steel wires with rubber stoppers, and fluoroscopically guided suture anchor placement with spiked washers, although these studies utilized human models over veterinary analogs (Motta, Errichiello et al. 1997; Fanter, Davis et al. 2012).

Barbed sutures are a relatively recent entry into the veterinary field, but have multiple uses in human medicine including flexor tendon repair, bariatric and cosmetic surgery procedures (Rashid, Sartori et al. 2007; Villa, White et al. 2008; Parikh, Davison et al. 2009; Greenberg 2010). Veterinary studies have demonstrated that knotless barbed sutures have

supraphysiologic load to failure, and in some cases, increased load to failure versus conventional sutures (Arbaugh, Case et al. 2013; Ehrhart, Kaminskaya et al. 2013; Nelson and Hassel 2014). These suture barbs are laid out in a helical fashion around the circumference and anchor in place without the need for a knot in both unidirectional and bidirectional styles (Villa, White et al. 2008). Each barb provides a component of total anchoring force which can exceed the tensile strength of the smooth monofilament sutures (Ingle and King 2010). The barbs increase tendon contact and may distribute load more evenly throughout the construct and minimize suture pull-out (Parikh, Davison et al. 2009). As these sutures are processed to a smaller core diameter, it is recommended both by the manufacturer and mechanical testing to select a barbed suture 1-2 sizes larger than the appropriate smooth suture, expecting at least equal tensile strength (Rashid, Sartori et al. 2007; Villa, White et al. 2008; Greenberg 2010). At the time of the study, there was no literature available on the potential feasibility of barbed sutures for canine Achilles repair.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Canine Achilles injury in veterinary medicine

Achilles injuries are most commonly reported in medium to large breed dogs (Carmichael and Marshall 2012). These injuries can occur during the course of normal activity, during play, or as a result of trauma. A predisposition to Achilles rupture has been noted in Dobermans, Labrador Retrievers, and overweight neutered bitches, with these ruptures most commonly noted at the insertion of the Achilles tendon to the calcaneus or the distal portion of the tendon (Reinke, Mughannam et al. 1993; Corr, Draffan et al. 2010; Gilbert, Shmon et al. 2010). Animals will often present initially with a non-weight-bearing lameness that may improve to some degree over a few weeks. This improvement is generally temporary, as the increased load on the remaining structures leads to a plantigrade stance over time (Corr, Draffan et al. 2010). If the superficial digital flexor tendon remains intact, there is an obvious “clawing” of the foot as the load placed on the intact tendon causes mechanical contraction of the digits without the protective influence of the gastrocnemius tendon (Fahie 2005; Corr, Draffan et al. 2010).<sup>2</sup>

A classification scheme was proposed to further describe Achilles injuries in the canine based on anatomy and gross pathology findings in affected animals (Meutstege 1993). This

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system categorized lesions into three types, with type 1 being the most severe, i.e. a complete rupture. Type 2 had three subtypes for partial rupture with an altered Achilles complex. Subtype A is defined as musculotendinous rupture, while B indicates rupture with intact paratendinous structure, and type C is a gastrocnemius avulsion with intact superficial digital flexor tendon. Type 3 is inflammation or irritation of peritendinous structures or the tendon itself (Meutstege 1993; Fahie 2005). Injuries can and do progress from initial classifications.

Diagnosis is made at the time of presentation with a thorough orthopedic examination. Patients with type 1 Achilles injury will have a plantigrade stance on the affected limb when bearing weight. The tibiotarsal joint can be flexed without flexing the digits. Tendon ends are often palpable in acute cases. Patients with type 3 injuries may have a normal stance with no flexion of the tibiotarsal joint when the stifle is extended. Type 2 injuries can occupy all points in between the other two types, but often have significant digital flexion when standing (Fahie 2005). Bilateral injuries are possible and abnormalities in gait or posture must be evaluated for concurrent neurologic abnormalities.

## 2.2 Connective tissue composition

Connective tissues are generally composed of a combination of cells, collagen, and ground substance. Collagen is an ever-present protein in the body, and uniformly associated with all healthy connective tissues. Ground substance is an amalgam of proteoglycans, afibrillar proteins, proteolipids, growth factors and glycoproteins. Glycoproteins are covalently bonded carbohydrate peptides. Proteoglycans are a subset of glycoproteins where the carbohydrate

components are unbranched polysaccharides containing sugars with an amino component, also known as glycosaminoglycans (Bliss, Rawlinson et al. 2012).

### 2.2.1 Collagen

Collagen is the most common protein in mammals, making up approximately 90% of the weight of desiccated dense fibrous tissues such as tendon (Bliss, Rawlinson et al. 2012). There are at least 28 subtypes of collagen, with the fibrillar collagens including the major subtypes (I, II, III), as well as several minor subtypes. Fibrillar collagen forms the majority of macromolecule collagen fibrils which can be noted microscopically in musculoskeletal tissues as groups of protein with regular arrangement in the extracellular matrix. Several associated collagen subtypes (IX, XII, XIV, XVI, XIX, XX, XXI, and XXII) help in three-dimensional orientation and interaction of the fibrillary collagen components. Other collagen subtypes include those that form the basement membrane and filamentous collagens (IV, VII, XV, and XVIII) (Bliss, Rawlinson et al. 2012). Regardless of subtype, all collagen shares a tertiary structure of a triple helix with three individual polypeptides known as alpha chains. These are encoded by unique genes and over 30 different genes are present in mammals. Some collagen subtypes, such as II and III, are composed of identical alpha chains and are referred to as homotypic. Other subtypes may have two or three different alpha chains forming their composition. The variety of other collagen subtypes come from the use of different transcription starting points or different splicing of primary alpha chains following transcription. The inherent variability associated with these alterations helps account for the high level of variability in the functional, as well as the structural and mechanical properties of these individual subtypes.

The synthesis of collagen starts with the transcription of the alpha chain and translation of the resulting alpha chain mRNA. This leads to the creation of pre-pro-collagen. Prolyl- and lysyl-hydroxylase activity causes hydroxylation of lysine and proline components at specific locations within the polypeptide at the same time as mRNA translation. Several cofactors are required to ensure this process, including oxygen, iron and ascorbic acid. An additional step that is vital to the formation of collagen is proline hydroxylation. This is both the primary rate-limiting step in collagen synthesis, as well as an essential part of the process, as mutations affecting this functional aspect lead to a particularly debilitating form of osteogenesis imperfecta (Marini, Cabral et al. 2010). The triple helix structure is built intracellularly and regulated by the C-terminal telopeptides of the pre-pro-alpha chains, then stabilized by disulfide bonds. The folding process itself is multifaceted, with cis-trans-isomerization of peptide bonds catalyzed by prolyl-peptidyl isomerase and other molecules providing additional guidance. This ultimately leads to the formation of the triple helix procollagen. Telopeptide regions are then removed by multiple types of metalloproteinases to allow the formation of tropocollagen; this process happening either directly before or shortly after secretion (Hulmes 2002).

Tropocollagen is built into complex fibrillary macromolecules in the extracellular matrix through a process known as fibrillogenesis. Tropocollagen molecules are staggered and cross-linked with covalent bonds to form fibrils by copper requiring lysyl-oxidases. These fibrils can vary in diameter from approximately 50 to several hundred nanometers. The assembly process is theorized to be largely based on the concept of entropy, based on the affinity of the hydrophobic tropocollagen terminals for avoiding the aqueous surroundings and intimately

associating with the interior of the fibrils under construction (Kadler, Hojima et al. 1987). Fibrils may be built or incorporated into larger constructs known as fascicles. Fascicles are further conglomerated into macroscopic fibers in tissues such as tendon, with diameters that can reach into the range of hundreds of micrometers.

The mechanical properties of collagen enriched connective tissues are not only based on their intrinsic mechanical properties, but also the three-dimensional structure, assembly, and diameter of the collagen fibrils themselves. A large variation is possible in the building of these collagens, leading to the ability to adapt the construct to the specific needs of a particular tissue. With regards to tendons, loads are primarily unidirectional and tensile in nature. Therefore, the parallel arrangement and higher collagen fiber density allow for enhanced stiffness in that direction.

### 2.2.2 Proteoglycans

Proteoglycans are an important component of the extracellular matrix that forms connective tissues. They consist of a centralized protein with one or multiple covalently bonded glycosaminoglycan chains peripherally attached. The central proteins are coded by various genes and reflect a large number of variations in both form and molecular sizes. The glycosaminoglycans are repeating disaccharide polymers of variable size. They can be divided into two main categories, glucosaminoglycans (heparan sulfate and keratan sulfate), which have D-glucosamine, and those containing D-galactosamine (chondroitin sulfate and dermatan sulfate) (Bliss, Rawlinson et al. 2012). Proteoglycans are typically divided into two large subtypes. The first consists of a hyaluronic acid spine and multiple linked proteoglycans that

attach through a linked protein that binds to the spine and the N-terminal area of the central proteoglycan protein. These large, coalescing proteoglycans include aggrecan and versican and their massive structures are significantly hydrophilic. This hydrophilicity greatly increases the compressibility of the tissue and is vital in tissues such as articular cartilage. The other subtype is a grouping of proteoglycans with high concentrations of leucine. The central proteoglycan proteins in this subtype have multiple attachment sites for conjugation of other molecules, including a variable setup for glycosaminoglycan attachment. These variations in structure and proteoglycan expression are specific to the individual tissues and help control collagen fibrillogenesis, elastogenesis, and help to alter the growth factor signaling to connective tissue cells (Ameye and Young 2002; Schaefer and Iozzo 2008).

### 2.2.3 Elastin and elastic fibers

Elastic fibers are present in varying amounts in different types of musculoskeletal tissues, making up the majority of the nuchal ligament, but less than 5% of the dry weight of tendon (Vrhovski and Weiss 1998). These fibers help prevent resistance to deformation under load while providing flexibility as a core component of the extracellular matrix. The genesis of elastic fibers is a large and active process during development, but turnover is almost non-existent in the adult (Petersen, Wagberg et al. 2002). The process is restarted following injury or after a prolonged tensile force is applied to a number of tissues, including tendon. These fibers are primarily made up of elastin, which is insoluble and extensible. The monomer component of elastin is tropoelastin, which is highly conserved in vertebrates. The tropoelastin transcript is subject to significant splicing after production, leading to the variety of forms and



functional abilities associated with tropoelastin as a whole (Indik, Yeh et al. 1989). The formation of elastin depends on availability of fibrillin microfibrils and fibronectin within the extracellular matrix to form a scaffold for tropoelastin deposition. If fibrillin is not present for elastogenesis, arterial wall defects and pathologic joint laxity, such as those noted in people suffering from Marfan's syndrome, are common. Elastic fibers are incredibly strain-resistant, with elastic deformation of approximately 70% of their initial length possible and a maximum extensibility of 220% before losing strength (Bliss, Rawlinson et al. 2012). Additionally, elastic fibers can be cycled billions of times through extension and relaxation without losing integrity, as they become more ordered under a load and relax into a state of higher entropy as the load is released (Keeley, Bellingham et al. 2002). Elastic fibers also play an important role in cell-to-cell signaling through surface integrins and elastin receptors. These signaling events can affect cellular survival, migration, and chemotaxis (Robert 2005).

#### 2.2.4 Other extracellular matrix components

Other proteins, glycoproteins and proteolipids form smaller percentages of the extracellular matrix. Fibrillins and latent transforming growth factor beta binding proteins interact with proteoglycans, collagen, and elastin to form structural components of connective tissues. These proteins also affect cellular receptivity and proteins like fibronectin that allow adherence to the cellular surface serve a vital role as a primary scaffold for the deposition of other extracellular matrix components (Bliss, Rawlinson et al. 2012).

### 2.3 Tendon composition

Tendons are collagen-dense fibers of connective tissue attaching muscles to bone, often encased in a sheath that minimizes friction during motion. These tendons serve as transducers of muscular contraction to the attached components of the skeletal system. There are several different forms of tendons common in mammals. Flattened tendons, known as aponeuroses, form a wide connection similar to a band or leaf of tissue that connect muscle to bones or other fascial structures. The fascia lata or fascia of the biceps femoris would be an example of an aponeurosis. Tendons may also exist as discrete, positional structures that transfer the forces generated by muscle contraction to effect motion of a joint. They can be complex in their orientations, be organized within special grooves, and change directions multiple times, such as the flexor tendons. Lastly, tendons may be able to store significant quantities of energy. The common calcaneal tendon, for example, has a higher elastic fiber content and ability to both store energy and enable elastic recoil than other tendons (Alexander 2002; Benjamin, Kaiser et al. 2008; Bliss, Rawlinson et al. 2012).

The tendon itself is made up of tenocytes, long fibroblastic cells that depend on collagen fibers alignment and have significant interconnections. The majority of the extracellular matrix is composed of tightly packed type I collagen, with smaller quantities of other subtypes, including types II, III, V, VI, IX, and XI (Bliss, Rawlinson et al. 2012). In general, the diameter of the collagen fibrils alternates between a larger and smaller diameter to increase the density of the collagen fibrils in total within a given cross-section. The larger fibrils provide greater stiffness and resistance to higher applied loads. The smaller diameter fibrils increase surface

area and provide more attachment points for cross-linking, allowing variation in viscoelastic properties between tendons. These fibers, regardless of relative diameter, are coiled within an unstressed tendon and undergo both elongation and significant alignment in response to applied tension. It is important to remember that these tendons are anisotropic, and the most impressive stiffness and strength is noted when they are loaded along their long axis. In contrast, transverse loading demonstrates comparatively poor stiffness and strength. Additionally, the speed at which the load is applied affects the response of the tendon, allowing it to behave as a viscoelastic structure as these coiled collagen fibers align in response to the applied load. Therefore, a smaller load applied over a relatively longer timeframe may lead to relaxation and creep. More sudden or rapid loading will eventually lead to fibrillary elongation and the resulting increase in shear forces directly affect the resulting relaxation and creep (Elliott, Robinson et al. 2003; Robinson, Huang et al. 2005). As the tendon is stretched past its yield point, the crosslinks between fibrils and the three-dimensional structures of collagen are permanently altered, leading to plastic deformation.

## 2.4 Tendon healing

Tendon healing is a slow process with a multitude of players involved at the microscopic level. Tenocytes produce a number of the substances mentioned previously, including elastin, proteoglycans, glycoproteins, and collagen to form an extracellular matrix (Fahie 2005). Tendon tensile strength is generally comparable to bone, and has a capacity to exceed the requirements of daily activity. The initial stage of the healing process demonstrates a significantly lower ultimate strength as the affected ends are remodeled (Mason and Allen

1941). These tendon ends have less ability to hold sutures and cannot appropriately respond to physiologic stress.

There are three phases of acute tendon healing common in veterinary patients. The initial inflammatory phase is characterized by erythrocytes and neutrophils. Within the first 24 hours, monocytes and macrophages are the predominant types of cells and phagocytic processes are highly active on damaged or necrotic cellular components. The changing vascular permeability allows influx of vasoactive and chemotactic compounds, eventually leading to the migration of tenocytes to the injury site and fibrinous bridging by day three (Gelberman, Vandeberg et al. 1985; Sharma and Maffulli 2006). This migration leads to the initiation of type III collagen synthesis (Murphy, Loitz et al. 1994). Collagen synthesis signals the beginning of the remodeling stage of healing, during which water and glycosaminoglycan concentrations are elevated (Murphy, Loitz et al. 1994). At 7-14 days, the effect of any gap at the repair site is highly visible, with collagen fibers beginning to span the cut edge in those with good apposition, while there is a more unorganized and proliferative response in those tendons with less optimal apposition. Larger gaps at the cut surface lead to prolonged fibrinous healing and less vascular ingrowth from within the tendon itself (Gelberman, Vandeberg et al. 1985). After approximately 3 weeks, the strength of the well-apposed repair increases in direct relation to the applied stress, with more significant increases noted in conditions of unrestricted use (Gelberman, Vandeberg et al. 1985). Following approximately six weeks, a modeling stage is initiated (Sharma and Maffulli 2006). Tissue conformations are altered to appropriately reshape prior to utilization, and with this, the relative cellularity and concentrations of collagen and glycosaminoglycans are reduced. The modeling phase is subdivided into consolidation and

maturation. Consolidation takes from weeks 6 to 10 and the cellular components of healing shift to a more fibrous nature. Tenocytes are still very active at this time and this period is when tenocytes and collagen fibers begin to orient along the lines of anatomically applied stress (Hooley and Cohen 1979). Collagen production shifts towards type I and away from type III during consolidation (Abrahamsson 1990).

After 10 weeks, the maturation subphase is initiated and the fibrous tissue is gradually changed into tendinous tissue over the course of a year with a decline in tenocyte metabolism and tendon vascularity as the timeframe progresses (Abrahamsson 1990). Tendon healing can occur with proliferation of tenocytes from epitenons and endotenons or by infiltration of cells from the tendon sheath and synovium itself. Endotenon-related tenocytes produce more mature collagen of larger size than epitenon-related tenocytes (Fujita, Hukuda et al. 1992). Initially, the epitenon is more active in producing collagen, but the ratio gradually shifts in favor of the endotenon over time (Ingraham, Hauck et al. 2003). It is thought that the proportionality of contribution may be related to the type of injury, presence of synovium, stress at the site, or local anatomy, but the exact mechanism is not completely understood (Koob 2002).

Specific modulators of tendon healing include matrix metalloproteinases (MMPs) and nitric oxide (Riley, Curry et al. 2002). In a murine model, the expression of MMP-9 and MMP-13 peaked in the first 1-2 weeks. MMPs 2, 3, and 14 increased afterwards until the end of the 4<sup>th</sup> week (Sharma and Maffulli 2006). Based on these results, it has been supposed that MMP-9 and MMP-13 are vital primarily in collagen degradation, while the others are involved in both degradation and remodeling. Nitric oxide is thought to help in tendon healing by encouraging

angiogenesis and vasodilation, with peak concentrations after 7 days (Murrell, Szabo et al. 1997). Inhibition of nitric oxide synthesis has been shown to result in decreased load to failure and cross-sectional area in murine Achilles tendons (Murrell, Szabo et al. 1997).

Tendons are commonly broken into vascular and avascular categories, the latter of which the Achilles belongs. Vascular tendons receive blood supply from surrounding tissues, including periosteum, muscle and connective tissue. Avascular tendons are surrounded by a synovial sheath and synovial fluid with some inherent vasculature, but do not receive the same level of support as vascular tendons (Fahie 2005). When examined as a single unit by angiography, the canine Achilles has an asymmetric distribution of blood vessels, with the calcaneal insertion having the highest vascular density, a finding distinct from the human literature (Theobald, Benjamin et al. 2005). The density decreases towards the mid-body of the tendon and begins to increase again towards the musculotendinous junction (Gilbert, Shmon et al. 2010). The distal third of the tendon receives blood supply from vessels exiting the calcaneus distal to, but entering at the level of the calcaneal insertion through the paratenon. These vessels arise from a caudal branch of the saphenous artery and end approximately 2-3 cm proximal to the calcaneus (Gilbert, Shmon et al. 2010). The distal caudal femoral artery branches and supplies the musculotendinous junction of the Achilles proximally. The mid-body of the tendon is supplied by additional branches of the caudal saphenous artery, but importantly, no vessels traverse the entire length of the tendon and the majority of the vascular supply of the canine Achilles is therefore segmental with minimal, if any, interconnection noted between the different portions of the tendon itself.

## 2.5 Tendon macroanatomy

The principle cells of tendons are tenocytes. These cells produce collagen of multiple types, elastin, glycoproteins and proteoglycans. The type I collagen fibers produced are generally found to align parallel to the long axis of the tendon itself and help provide structural stability and strength (Maffulli and Kader 2002; Fahie 2005). In sheathed tendons, such as the Achilles, both an inner visceral layer and outer parietal layer of sheath are present. The outer layer is attached to the adjacent tissues, either connective tissue or periosteum as appropriate (Fahie 2005). These paired layers are joined by the mesotendon, an important structure in establishing neurovascular supply of sheathed tendons (Harari 1993). The tendon itself is further divided into three components, known as the endotenon, paratenon, and epitenon, respectively. The endotenon is comprised of type III collagen fibers that aid in forming neurovascular supply by forming smaller tendon subunits. The paratenon is loose areolar tissue within the tendon itself, and the epitenon is the connective tissue sheath surrounding the tendon itself (Paavola, Kannus et al. 2002; Fahie 2005).

## 2.6 Goals in healing

In both veterinary and human medicine, the primary objective of Achilles repair is to provide adequate tensile strength during tendon healing (Motta, Errichiello et al. 1997; Moores, Owen et al. 2004; Fahie 2005). Tendon heals slowly, only achieving 56% of initial strength at 6 weeks, and 79% at 1 year post suture repair (Dueland and Quenin 1980). Therefore, a rigid repair that will retain its strength over a prolonged period of time is necessary to allow the tendon to heal undisturbed. Retaining the gliding function of these tendons is a less important

secondary goal as the relative lack of digital dexterity is quite significant in veterinary patients when compared to their human counterparts (Harari 1993; Moores, Owen et al. 2004; Fahie 2005). Tendon healing is likely a complex process, with two main theories noted in human literature. The first is extrinsic healing, where the tendon relies on formation of adhesions, the inflammatory response and vascular supply from outside the tendon itself. The second theory is more intrinsic, supposedly relying on growth of epitenon and endotenon with no adhesions. The intrinsic theory is also based on an intratendinous vascular supply (Lin, Cardenas et al. 2004; Fahie 2005). At this time, there is no consensus as to which of these theories is most likely, nor is it definite that they are mutually exclusive.

## 2.7 Improving the healing process

Decreased tensile strength of Achilles repair has been associated with gap formation at the surgical repair site. The formation of the gap is indicative of increased strain at the repair site which significantly affects the progression of healing. Scar tissue proliferates at the repair site and rather than the preferred type I collagen, the more elastic type III collagen is produced (Fahie 2005). A gap of 3mm or less has been associated with a significantly increased load to failure, a decreased strain, and increased rigidity of tested tendons at six weeks post-operatively (Gelberman, Boyer et al. 1999; Lister, Renberg et al. 2009). These lesser gaps at the repair site have led to an increased endotenon response with better vascular ingrowth while the larger gaps healed with more of an epitenon response composed of increased fibrous tissue and requiring more time to reach comparable vascularization during the first two weeks post-operatively (Gelberman, Vandeberg et al. 1985; Fahie 2005).



Post-operative immobilization of surgically repaired Achilles injuries is a subject that is controversial in the literature. The minimization of strain at the repair site will allow the more beneficial type I collagen to form and align parallel to the long axis to increase overall strength. However, complete immobilization for longer than 21 days led to a significant decrease in vascularity and repair strength at the repair site (Gelberman, Menon et al. 1980; Woo, Gelberman et al. 1981). While the underlying mechanism is poorly understood, it has been proposed that the repeated mechanical loading of canine ligaments in a controlled manner alters the metabolic events associated with tissue repair and collagen synthesis (Gelberman, Woo et al. 1982). Controlled mobilization of repairs have been able to show similar load to failure at 9 weeks as fully immobilized repairs demonstrated at 12 weeks post-operatively (Woo, Gelberman et al. 1981). It is important to note that the majority of the studies regarding strain at the tendon repair site have involved digital flexor tendons, not the Achilles itself. Biomechanical study involving the Achilles has demonstrated that immobilization of the tarsus does not significantly change the maximum strain when compared to non-immobilized joints (Lister, Renberg et al. 2009). However, the ultimate tensile strength of tendons without a gap or with a gap <3mm is increased with improved healing at 6 weeks, compared to those with gaps larger than >3mm (Gelberman, Boyer et al. 1999; Boyer, Gelberman et al. 2001). Given this clinically significant difference, a general recommendation of immobilization or protection of the repair for 2 to 12 weeks has been recommended to attempt to further minimize gap formation (Lister, Renberg et al. 2009; Carmichael and Marshall 2012). Current methods for immobilization include transarticular external skeletal fixator, calcaneo-tibial bone screw, and

multiple configurations of casts and splints (Morshead and Leeds 1984; Reinke, Mughannam et al. 1993; De Haan, Goring et al. 1995; Guerin, Burbidge et al. 1998)

## 2.8 Fundamentals of Achilles repair

### 2.8.1. Material properties

During the weight-bearing phase of the canine pelvic limb, the gastrocnemius tendon bears the highest proportion of the load (Jopp and Reese 2009; Corr, Draffan et al. 2010; Gilbert, Shmon et al. 2010). With an Achilles injury, the repair would therefore be loaded in tension, with forces distracting the anastomosed repair on either side. Both static and cyclic loads will be placed upon the repaired tendon through body weight, muscular forces, and the impact of activity. This ability to retain strength in the face of these forces during the tendon healing process is vitally important. The ability to resist tensile forces is measured with a load-displacement curve. Each curve demonstrates elastic, plastic, and unstable deformation phases. Any deformation noted in the elastic region will resolve and the material will return to its original dimensions. The yield point separates the region of elastic deformation from that of plastic deformation. Plastic deformation will not allow the material to return to its initial state. Plastically deformed material will go back to a neutral or zero state in terms of applied load, but the shape will have permanently altered (Roeder 2013). After reaching the ultimate tensile strength or ultimate load, the deformation enters the unstable deformation phase. Deformation becomes unpredictable as the cross-sectional area of the material decreases with continued loading (Roeder 2013).

Tendon is a viscoelastic material, meaning that there is a time dependent part of the inherent material response to testing. The collagen, proteins, and ground substance of the intact tendon interact under load and are responsible for this characteristic load-deformation curve (Roeder 2013). When strain is applied to produce a constant deformation, slow relaxation is noted that is demonstrated with increasing deformation with proportionally lesser increases in load, resulting in a shallower slope on the load-deformation curve. However, if the strain is applied quickly to produce maximal initial deformation, the slope of the load-deformation curve will increase as a result (Figure 1) (Abrahams 1967; Roeder 2013; Lakes 2014).

#### 2.8.2 Suture repair

A multitude of suture repair patterns have been utilized in the clinical setting with variable success rates (Tomlinson and Moore 1982; Corr, Draffan et al. 2010; Carmichael and Marshall 2012). The simplest of techniques is a series of simple interrupted sutures. While technically the easiest repair pattern to place, there have been several issues with its use. In one early study, while the placement of multiple simple interrupted sutures had no significant difference in load applied to form a gap at the repair when compared to other methods, each and every sample failed with intact suture repairs rupturing through the tendon, causing further damage to the tendon (Pijanowski, Stein et al. 1989). Based on this finding, other suture patterns that engage more of the tendon to provide a better anchor for continued apposition of the tendon ends were recommended. A modification of the simple interrupted suture pattern, the Mason-Allen suture, depends on knotted anchor points on each side of the

tendon. This pattern demonstrated lesser performance in overall strength and resistance to gap formation than the simple interrupted pattern, while still lacerating tendon with intact suture material; it has largely fallen out of favor (Pijanowski, Stein et al. 1989).

The Bunnell, or Bunnell-Mayer, suture pattern is a more complex pattern that had been previously utilized for tendon repair. It involves multiple passes in a diagonal pattern on both sides of the repaired tendon. A more moderate tendency for gap formation was noted with this pattern than the Mason-Allen (Pijanowski, Stein et al. 1989). However, while better able to resist gap formation, the Bunnell-Mayer pattern results in significant tendon constriction and adversely affects the underlying vasculature (Tomlinson and Moore 1982; Pijanowski, Stein et al. 1989; Montgomery, Barnes et al. 1994; Moores, Comerford et al. 2004). Based on the perceived impairment to healing that this constriction might provide, the Bunnell-Mayer suture is also rarely used in the clinical setting at this time.

Due to the lackluster performance of these suture patterns in tensile testing, the modified Kessler, also referred to as the Kessler-Mason-Allen or locking loop suture pattern, was recommended for further testing and has been tested in single, double and triple loop configurations (Tomlinson and Moore 1982; Berg and Egger 1986; Easley, Stashak et al. 1990). The pattern is started on one side of the tendon with a longitudinal pass from the cut surface and a second superficial pass transversely to reach the other side of the tendon. This pattern is repeated in the reverse order to allow passage to the other half of the cut surface. In this fashion, a cloverleaf-type pattern is made and the ends secured on the same side with a knot. This pattern has several advantages to the Bunnell-Mayer suture, including lessened gap

formation, quicker placement of the repair, and avoiding interference with the intrinsic blood supply (Tomlinson and Moore 1982). This suture pattern is currently used within the clinical setting for Achilles repair based on clinician preference.

The three-loop pulley (3LP) is another pattern that has found more consistent usage in the clinical setting. It involves a series of three loops, each offset by 60° during placement of each pass, leading to a three-dimensional configuration when placed and a recommendation for use in round tendons (Moores, Owen et al. 2004). When utilizing this pattern, six strands of suture cross the repair site, as compared to two when a single locking loop is placed. Based on the proposed mechanical advantage this would provide, the 3LP suture pattern has been compared to locking loop patterns in canine tendon, and the 3LP suture pattern has consistently demonstrated greater tensile strength and less gap formation (Berg and Egger 1986; Moores, Owen et al. 2004).

Krackow, continuous cruciate, and far-near-near-far sutures have also been evaluated in tendons, but biomechanical comparisons to other patterns in the Achilles tendon are lacking or prove inferior when compared to locking loop patterns (Jassem, Rose et al. 2001; Renberg and Radlinsky 2001; Fahie 2005). Recently, the 6-strand Savage suture pattern was tested favorably against the 3LP pattern in equine superficial digital flexor tendon, but there is no data available on the gastrocnemius component of the Achilles and the ability to translate this information to canine tendons is unknown (Everett, Barrett et al. 2012).

### 2.8.3 Suture type

With regard to repair techniques, materials utilized fall under either absorbable or non-absorbable categories. The definition of absorbable is not universally agreed upon, but a commonly accepted meaning is a material that loses a majority of its tensile strength within 60 to 90 days within mammalian tissue (Chu 1997). Based on the prolonged need for load sharing within any Achilles repair, losing 51% or more of tensile strength within a 60 to 90 day period would be suboptimal for aiding in tendon healing and minimizing gap formation at the repair site. As such, absorbable materials are not commonly utilized for primary Achilles tendon repair. Common non-absorbable suture materials include silk, nylon, polyesters, stainless steel, and polypropylene. Of these choices, stainless steel has poor handling characteristics and manipulation may create stress risers that predispose repairs to failure (Chu 1997). Silk is a natural non-absorbable material that loses 56% of its initial tensile strength within 12 weeks (Greenwald, Shumway et al. 1994). Of the remaining options, polypropylene has demonstrated the highest energy requirement to breakage (Kim, Lee et al. 2007). Polyester multifilament composite sutures, sometimes referred to as polyblends, are options that improve breaking strength and resistance to fray. These polyblend sutures are becoming more common in both human and veterinary medicine.

#### 2.8.3.1 Polypropylene

Polypropylene is a polymer of propylene, one of the simpler members of the alkene family, also commonly referred to as an olefin. Propylene is polymerized with the use of metal and metal alkyl catalysts to produce a polymer with commercial value (Malpass and Band 2012). The degree of polymerization is generally greater than 1000 for most commercially

available forms of polypropylene. Structural components of the monomer have arrangements that can lead to a variety of three-dimensional structures, and various forms of “tacticity” exist (Malpass and Band 2012). When the methyl groups are oriented in the same direction, the polymer is isotactic, the Greek term for “ordered” (Malpass and Band 2012). This is the most common form of industrial polypropylene, has a very large crystalline component, and a three-dimensional helical structure owing to the most efficient way to pack the protruding methyl groups. The greater the variation in the location of the methyl groups, the more amorphous the material becomes, leading to poorer structural characteristics. As the extent of crystallization increases, the strength, stiffness, density and melting points all increase (Maier and Calafut 1998).

Polypropylene suture is generally produced by extrusion and subsequent biaxial drawing (Liu and Brewer 1993; Maier and Calafut 1998). The polypropylene is heated up to a temperature just below its melting point, which can vary from 160-166° Celsius for available isotactic varieties and extruded into the fibers that will become the suture (Maier and Calafut 1998; Malpass and Band 2012). Full liquefaction would immediately and drastically alter the mechanical properties of the polymer and is avoided during processing. After extrusion, the polypropylene fibers are partially quenched to allow them to further solidify prior to drawing. The material is drawn at elevated temperatures multiple times to increase its fiber orientation and tensile strength before annealing, leading to an ultimate elongation of the produced polymer fibers by 35 to 63 percent in some products (Liu and Brewer 1993). This increase in fiber orientation through biaxial drawing leads to an increase in tensile strength, the elastic modulus, drop impact strength, gloss, and shrinkage. It also leads to a decrease in total

permeability (Maier and Calafut 1998). The gains in strength and relative impermeability after processing increase the polypropylene fibers suitability for usage in medical applications such as wound closure.

## 2.9 Barbed Suture

Barbed sutures were developed for, and initially found a high usage rate in facial suspension procedures (Villa, White et al. 2008). These sutures have radial projections, often arranged in a helical fashion, that allow for supplemental tissue anchorage points. These projections, commonly referred to as barbs, are cut into the surface of the polymer suture around the suture core (Villa, White et al. 2008). When placed into tissues, the barbed sutures are passed into the tissue at the narrow apex of the projection, allowing passage of the suture without engaging the barbs as anchor points. When pulled in the opposite direction, the barbs splay outward and prevent further motion in that direction (Villa, White et al. 2008; Parikh, Davison et al. 2009; Ingle and King 2010). This characteristic splaying of the barbed anchors eliminates the need for knots to secure the sutures and resist tensile forces when placed (Leung 2004).

The manufacturing process of barbed sutures involves cutting the surface of a smooth suture to form the barbs helically around the length of the suture core (Leung 2004; Rashid, Sartori et al. 2007; Villa, White et al. 2008). As the machining process decreases the core suture diameter, it has been demonstrated that suture strength of barbed suture is comparable to that of smooth suture one to two sizes smaller in diameter (Rashid, Sartori et al. 2007; Villa, White et al. 2008; Parikh, Davison et al. 2009; Greenberg 2010).



### 2.9.1 Directionality

Barbed sutures are commonly produced in both unidirectional and bidirectional configurations. The bidirectional suture has a central unbarbed portion in between helical arrays oriented 180 degrees in opposition to each other as they travel to the ends of the suture, each with a swaged-on needle. In veterinary medicine, the unidirectional suture (V-LOC, Covidien Inc., Minneapolis, Minnesota, USA) is secured per manufacturer's recommendations after performing the initial suture pass. A loop is present on the end of suture opposing the needle and after passing the needle through both sides of the site to be closed at one end of the wound, the suture is passed through the loop to secure the pass before continuing on in the desired fashion. Repair with the bidirectional suture (Quill SRS, Surgical Specialties Co., Vancouver, British Columbia, Canada) is initiated at the midpoint of the wound and passing one arm of the suture through the site until the barbs facing the opposite direction are engaged. This process is repeated with the other suture arm, and per manufacturer's recommendations, once at least two suture bites are taken on each side, the suture may be tightened as appropriate (Angiotech 2013). Neither the unidirectional nor the bidirectional suture requires a knot to terminate the pattern, as the barbs themselves provide the total anchoring force.

### 2.9.2 Barbed Suture Materials

Barbed sutures can be produced by micromachining most synthetic monofilament sutures, including polydioxanone, polyglyconate, poliglecaprone 25, glycomer 631, nylon, and polypropylene (Leung 2004; Rashid, Sartori et al. 2007; Greenberg 2010). Polypropylene is the most commonly used synthetic monofilament barbed polymer suture utilized for several types

of human cosmetic procedures (Rashid, Sartori et al. 2007). As such, multiple studies are available to establish baseline material properties for barbed polypropylene suture and allow for more appropriate experimental comparisons (Rashid, Sartori et al. 2007; Villa, White et al. 2008; Ingle and King 2010). Multiple modifications have been proposed for alteration of the cut depth and angle utilized to produce the barbs in polypropylene suture, but as of this time, the ideal combination for tissues such as skin, tendon and fatty tissue are unknown (Ingle and King 2010).

## CHAPTER 3

### MATERIALS AND METHODS

#### Objectives

The aim of this study was to determine if knotless polypropylene barbed 3LP gastrocnemius repair results in superior load to failure and reduced gap formation when compared to smooth knotted suture when evaluated in a single tensile test to failure. Stronger repairs may reduce the need for external support and minimize associated complications. We hypothesized that knotless barbed polypropylene suture in a 3LP for *in vitro* repair of the gastrocnemius tendon would have higher UTS and less gap formation compared to smooth knotted polypropylene suture under these conditions.<sup>3</sup>

#### Sample preparation

Common calcanean (Achilles) tendons and calcanei were obtained as a paired unit from the pelvic limbs of adult, sexually intact mixed breed dogs euthanized for unrelated reasons and free of gross orthopedic disease. All soft tissues, except for the gastrocnemius tendon, were removed. Each bone-tendon unit was wrapped in saline-moistened towels (0.9% NaCl) and stored at -20°C until testing. The units were thawed to room temperature overnight prior to testing. The calcaneus was secured in a vise (Tool Shop Multi-Angle Drill & Vise Clamp, Menard, Inc., Eau Claire, Wisconsin, USA) at 135° to mimic the standing angle of the canine hock. This

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<sup>3</sup> This chapter contains previously published material from: Perry, B. S., T. A. Harper, et al. (2014). "Barbed versus smooth polypropylene three-loop pulley sutures for repair of canine gastrocnemius tendon." *Vet Comp Orthop Traumatol* **27**(6): 436-440. Reproduced here for educational/teaching purposes only with permission of the publisher.

vise was secured by proprietary grip faces (MTS Serrated Screw-Action Grip Face Surface #056-163-803, MTS, Inc., Eden Prairie, Minnesota, USA) and grips (MTS Advantage Screw Action Grips #554268-01, MTS, Inc., Eden Prairie, Minnesota, USA) within a tensile testing machine (MTS Insight 2, MTS, Inc., Eden Prairie, Minnesota, USA). The distance between the clamp and the calcaneus was zeroed prior to each test. The proximal part of each tendon was secured in a second set of grips (Figure 2).

### Mechanical testing

Tensile testing was performed with a servohydraulic testing machine (MTS Insight 2, MTS, Inc., Eden Prairie, Minnesota, USA) equipped with a 2000 N load cell. Tendons were preloaded to 2 N for precise transection. The gastrocnemius tendon was transected with a #11 scalpel blade, 3 cm proximal to the tuber calcanei, and repaired with a 3LP. One of each pair was randomly selected by coin flip to receive repair with 3-metric smooth monofilament polypropylene suture (Prolene, Ethicon Ltd., San Angelo, Texas, USA ) [SP], and the other was repaired with 4-metric bidirectional barbed monofilament polypropylene suture (Quill, Angiotech Pharmaceuticals Inc., Vancouver, British Columbia, Canada) [BP]. For the 3LP, the near bite was 5 mm, the middle bite 10 mm, and the far bite 15 mm from the anastomosis site, as described by Moores, et al (Moores, Comerford et al. 2004). Each loop was placed 60° around the tendon circumference to the previous loop. The SP repair was secured with a surgeon's throw and two square knots after anatomic apposition of tendon ends (Moores, Owen et al. 2004). The BP repair was performed in two parts. Three suture passes were performed in one direction, passing the first arm of suture through the tendon until the barbs

directed 180° from that arm engaged the tendon. The last three passes were performed with the second arm in the opposite direction (Figure 3). The primary author performed all repairs. Ink was placed at the cut surfaces of the tendon with a permanent marker (Sharpie Fine Point Permanent Marker, Newell Rubbermaid Office Products, Oak Brook, Illinois, USA) after tensioning again to 2 N. Repaired tendons were tensioned to suture breakage or point of tissue pull-through at a linear distraction rate of 25 mm/min. Each test was filmed with a digital video camera (JVC GZ-MG680BUS, JVC Americas Corps., Wayne, New Jersey, USA) focused on a metric ruler adjacent to the tendon for evaluation of gap formation. Load-displacement data was collected from tensile testing software (MTS TestWorks 4, MTS Inc, Eden Prairie, Minnesota, USA) and acquired at 10 Hz. The camera allowed simultaneous readout of the tensile testing display, a secondary method of data acquisition. Video data allowed evaluation of mode of failure, grip slippage, and quantification of gap formation. The load-displacement curve generated by the software determined the UTS, as well as the load required to produce 1 and 3 mm gap.

#### Data analysis

*A priori* power analysis was performed to determine sample size using the following criteria: an alpha of 0.05, a power of 0.8, and an expected mean difference of 3 N with a standard deviation (SD) of 4 N between groups. Utilizing these parameters, a minimum sample size of 28 tendon constructs per group was determined. Statistical analysis was performed with commercial software (SPSS Statistics Software version 22, IBM Inc, Armonk, New York, USA). For all tendon units, data points for load (N) at 1 mm gap, 3 mm gap, and UTS were recorded.

The distribution of data was analyzed for normality and variance with Kolmogorov-Smirnov testing. Levene's Test was used to evaluate the equality of variances between groups. Independent-samples T-tests were utilized for comparing the outcomes associated with repair material. A Bonferroni correction was applied to minimize family-wise error. A  $p < 0.017$  was used to determine statistical significance. Reported load values were recorded as mean values with SD.

## CHAPTER 4

### RESULTS

#### Loads at failure, gap, and ultimate tensile strength

Bone-tendon units were obtained from 16 female and 17 male dogs with an average weight of 21.8 kg (SD: 3.2, range 16.8-27). There was a significantly higher ( $t=5.310$ ,  $p<0.001$ ) UTS for SP ( $40.5 \pm 10.6$  N) compared to the BP repairs ( $28.6 \pm 7.1$  N). The load resulting in a 1 mm gap for the BP ( $17.2 \pm 5.3$  N) was less ( $t=6.202$ ,  $p<0.001$ ) than the SP repairs ( $26.8 \pm 7.1$  N). The 3 mm gap load for the BP ( $25.3 \pm 5.7$  N) was also significantly lower ( $t=4.985$ ,  $p<0.001$ ) than the SP repairs ( $35.1 \pm 9.4$  N) (Figure 4, 5).<sup>4</sup>

#### Methods of failure

Thirty-one BP repairs (94%) failed when barbs reversed direction and suture backed out. Two (6%) BP sutures broke under tension at the most proximal placement point. Neither of these sutures experienced barb reversal. Thirty-one SP (94%) failed by suture material pulling through tendon. In all cases, knot and suture material remained intact. The remaining two constructs (6%) failed by tendon tears proximal to the repair at the clamp-tendon interface.

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<sup>4</sup> This chapter contains previously published material from: Perry, B. S., T. A. Harper, et al. (2014). "Barbed versus smooth polypropylene three-loop pulley sutures for repair of canine gastrocnemius tendon." *Vet Comp Orthop Traumatol* **27**(6): 436-440. Reproduced here for educational/teaching purposes only with permission of the publisher.

## CHAPTER 5

### DISCUSSION

The results of this study demonstrate that the load at each sample point (1 mm gap, 3 mm gap, UTS) was significantly higher for the SP repair. In regards to the suture pattern selected, the 3LP is an accepted and widely utilized technique for canine tendon repair (Moore, Owen et al. 2004). While there are other techniques that may be superior to the 3LP, there is a lack of baseline data in canine tendons (Everett, Barrett et al. 2012). The BP repair pattern used in this study mimics the traditional 3LP and minimizes the influence of directionality of forces. As noted in a previous biomechanical study, the barbs should allow more even load distribution throughout the repair due to an increased total surface area of barb-tendon interface (Parikh, Davison et al. 2009). The 60° offset and orientation of each suture pass prevents bias towards a single barb orientation and helps prevent stress concentration in a single plane, as would occur if all passes were oriented in the same direction (Ingle and King 2010). By testing in a manner similar to a previously reported, biomechanically tested, and commonly used smooth knotted polypropylene repair, a true baseline was established for the barbed polypropylene suture (Moore, Owen et al. 2004). Variability was further minimized by restricting testing to the gastrocnemius tendon, the major load-bearing component of the Achilles.<sup>5</sup>

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## Materials and methods considerations

Ideally, suture material used for tendon repair should be strong, inert and share load until complete healing. Size 3-metric SP suture is utilized by the authors' clinic for this injury for dogs in this weight range. The 4-metric BP was utilized based on published recommendations to increase one to two suture sizes versus a smooth suture equivalent (Rashid, Sartori et al. 2007; Villa, White et al. 2008; Greenberg 2010).

This study focused on tensile testing to failure as a single test. Cyclic testing under physiologic loads would be more representative of *in vivo* conditions, but these results give significant insight. Ground reaction force of a pelvic limb in a standing dog is approximately 20% of body weight, 31 – 54 N in this study (Phelps, Ramos et al. 2007). Assuming stance phase during trotting allows 65-70% of body weight to pelvic limb peak vertical force, one can expect a maximum value of 187 N for the dogs in this study (Rumph, Lander et al. 1994; Budsberg, Rytz et al. 1999). Moores et al. demonstrated that the 3LP with SP should be reinforced with supplemental support, and this study showed that BP was significantly weaker than that repair (Moores, Owen et al. 2004). Therefore, the data does not support BP 3LP repair as equivalent or superior to the SP.

## Failure mode

Two of the BP sutures broke during tensile testing while the remaining specimens failed by suture pull-out. In these specimens, the same single suture pass pulled out prior to failure, resulting in a 3 mm gap between the cut tendon surfaces in each test. On examination of the suture, barb reversal was present. This may be due to barb realignment within that suture

pass in the loaded tendon. A primary mode of failure in barbed suture pullout testing is barb bending, or reversal, where a segment of barbs fails to anchor adequately in tissue and facilitates pullout by bending backwards without any further damage to either the suture or the surrounding tissues (Ingle and King 2010). The barbs remaining within the repair could not be assessed without removal from the tendon, potentially changing their conformation prior to evaluation and preventing accurate assessment of barb orientation at the time of failure. One study, in which barb reversal was reported following facial suspension procedures, demonstrated that pulling new barbed suture through bovine muscle did not result in alteration of barb orientation (Helling, Okpaku et al. 2007). Barbed suture that had already been loaded was not tested. Therefore, we cannot draw any definitive conclusions about reversal of barb orientation after pulling loaded suture out of the tendon. The conformation of the barbed suture utilized in this study caused an interesting tendency in the load-deformation curves. Even with the single suture pass pull out, the BP repairs retained an ability to resist further load at a lower level, resulting in at least three peaks on the load- deformation curves. With the SP samples, 94% failed by intact suture pulling through tendon. In each, the suture seemed to momentarily catch and hold its position closer to the cut surface prior to failure. These SP repairs demonstrated elastic and plastic deformation prior to failure at each position.

#### Barb reversal

To the authors' knowledge, the barb reversal in the BP is the first time this failure method has been seen in veterinary studies (Arbaugh, Case et al. 2013; Ehrhart, Kaminskaya et al. 2013; Nelson and Hassel 2014). Biomechanical testing of barbed suture has relied on knots

in *ex vivo* testing, and studies demonstrating at least comparable strength of the repair do not utilize the 3LP (Arbaugh, Case et al. 2013; Ehrhart, Kaminskaya et al. 2013; Spah, Elkins et al. 2013; Nelson and Hassel 2014). This study represents the first time that knotless barbed suture has been used to repair a significant load-bearing canine tendon and has no direct comparison in the literature. There were several limitations to this study. The barbed repair should increase the cumulative construct holding strength through an increased barb-tendon interface, as noted when knotted prior to testing in previous studies (Parikh, Davison et al. 2009; Ingle and King 2010). However, this did not bear out in the results for any of the load values, likely due to barb reversal of the knotless repair noted prior to the UTS of the SP.

At the time of study completion, this had been the first time that the use of knotless barbed sutures has been reported for primary orthopedic repair in veterinary surgery. Since completion of this testing, a study in human Achilles tendons has demonstrated a lower peak load to failure in barbed suture compared to a conventional repair, similar to our results (Kanz, Morris et al. 2014). While other human studies in animal models have shown promising initial results and veterinary studies have demonstrated that knotless barbed sutures have at least comparable load to failure in selected soft tissue closures, further testing is warranted.

## CHAPTER 6

### CONCLUSIONS

The present study allowed us to conclude:<sup>6</sup>

- Bidirectional barbed polypropylene suture can be successfully placed in a knotless modified 3LP suture pattern within a canine gastrocnemius tendon.
- Bidirectional barbed polypropylene suture placed in a knotless modified 3LP suture pattern had a significantly lower ultimate tensile strength when compared to appropriately sized smooth polypropylene placed in a standard 3LP suture pattern.
- Bidirectional barbed polypropylene suture placed in a knotless modified 3LP suture pattern had a significantly lower load to produce a 1 mm gap when compared to appropriately sized smooth polypropylene placed in a standard 3LP suture pattern.
- Bidirectional barbed polypropylene suture placed in a knotless modified 3LP suture pattern had a significantly lower load to produce a 3 mm gap when compared to appropriately sized smooth polypropylene placed in a standard 3LP suture pattern.
- The majority (94%) of bidirectional barbed polypropylene suture repairs failed by barb reversal, a previously unreported failure method in veterinary studies.

The testing performed in this study is not representative of *in vivo* conditions, as the soft tissues surrounding the gastrocnemius tendon were stripped away to minimize variability in

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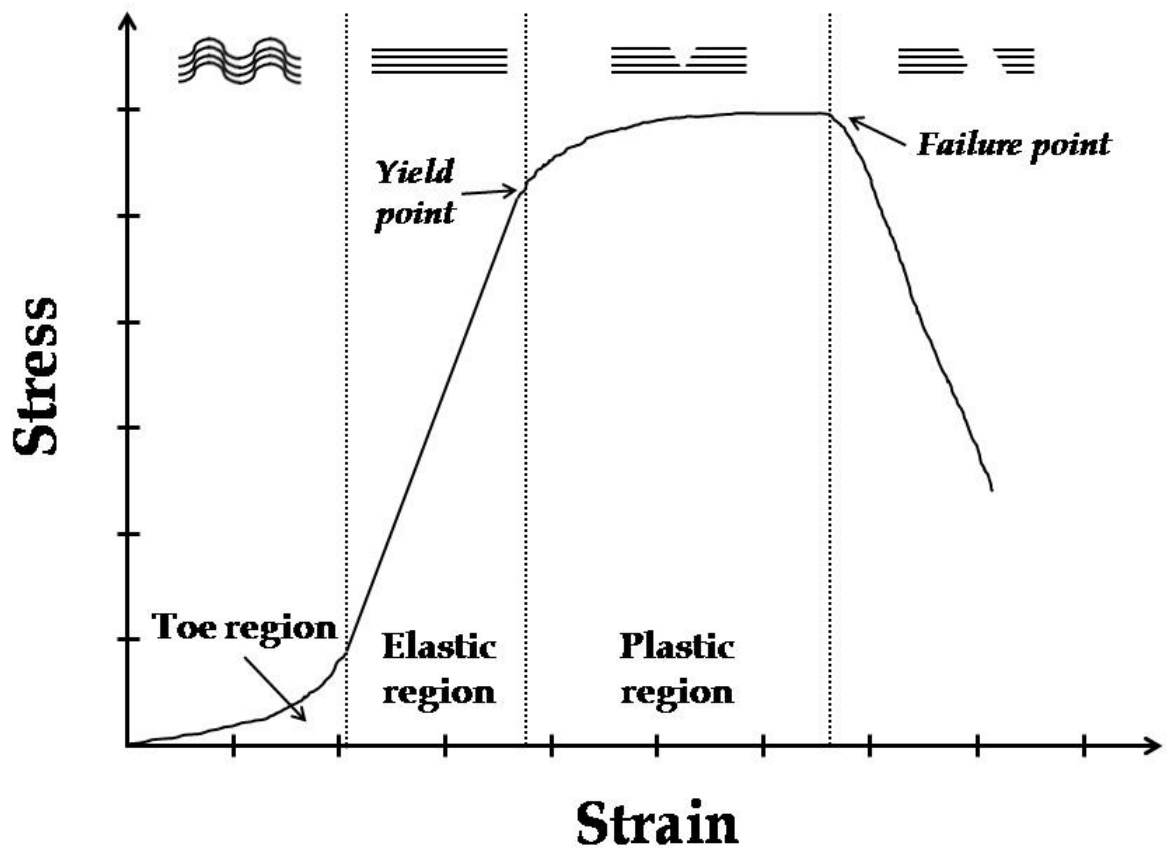
<sup>6</sup> This chapter contains previously published material from: Perry, B. S., T. A. Harper, et al. (2014). "Barbed versus smooth polypropylene three-loop pulley sutures for repair of canine gastrocnemius tendon." *Vet Comp Orthop Traumatol* **27**(6): 436-440. Reproduced here for educational/teaching purposes only with permission of the publisher.

the testing conditions. These tissues do provide some degree of supplemental support in the live animal and could not be assessed with this study design. As this was a cadaveric study, the effect of progressive healing on the repair cannot be assessed. Finally, as was stated earlier, a cyclic loading test would be more representative of *in vivo* conditions. However, the results of a single load to failure are statistically significant and we cannot recommend moving forward with cyclic load testing of the bidirectional barbed polypropylene suture placed in a knotless modified 3LP suture pattern as currently devised.

## CHAPTER 7

### FIGURES

Figure 1. Graphical representation of typical load-deformation curve of intact tendon. The images at the top of the graph represent collagen fiber orientation in each region of the graph with gap formation noted to begin in the plastic region of deformation (Rami and Simo 2011).<sup>7</sup>



<sup>7</sup> This chapter contains previously published material from: Perry, B. S., T. A. Harper, et al. (2014). "Barbed versus smooth polypropylene three-loop pulley sutures for repair of canine gastrocnemius tendon." *Vet Comp Orthop Traumatol* 27(6): 436-440. Reproduced here for educational/teaching purposes only with permission of the publisher.

Figure 2. Photograph of servohydraulic testing apparatus prior to tensile testing. The white arrow indicates the level of tendon transection and ink placed at both cut surfaces.



Figure 3. Diagram of bi-directional 3-loop pulley (3LP) suture pattern. The points designated with 1a and 2a represent the starting point of each arm of suture, which are different shades to differentiate them further. The arrows on the suture demonstrate the direction of suture passage as well as barb conformation.

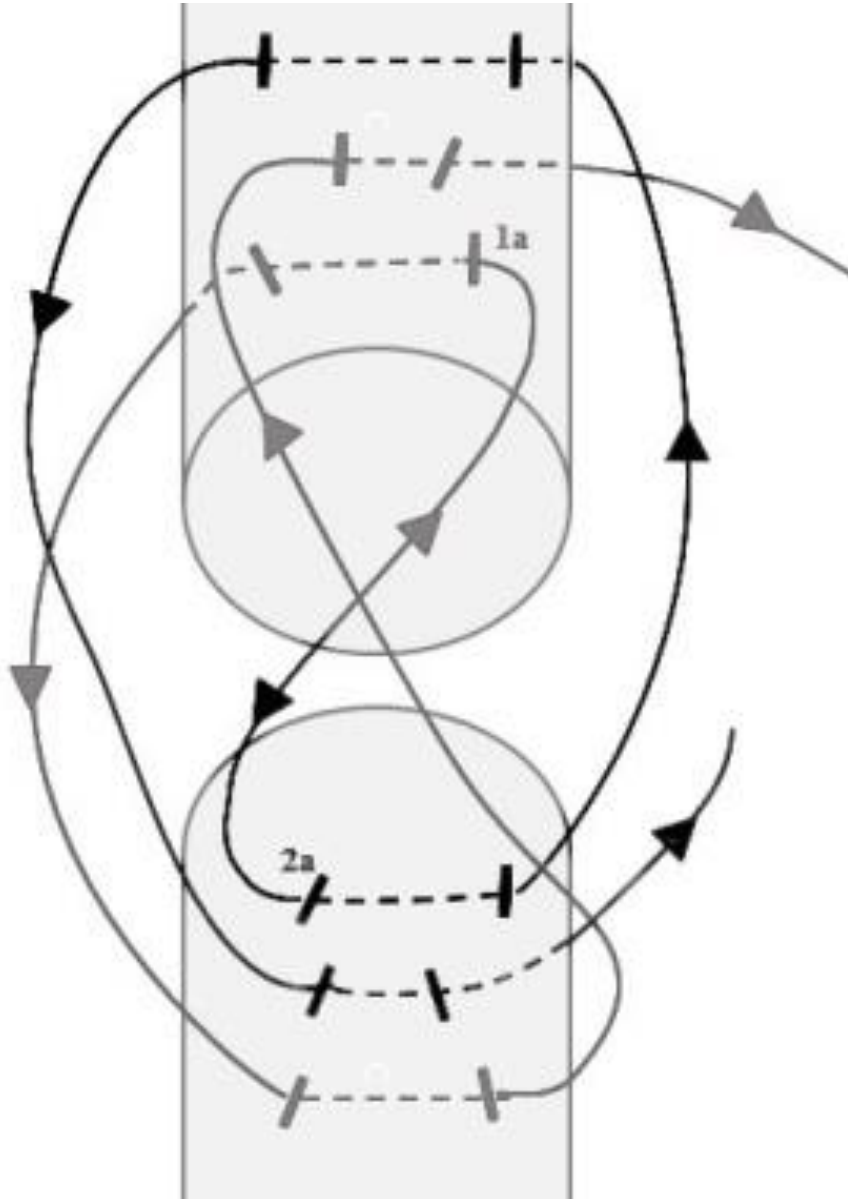




Figure 4. Representative sample results for load-displacement curve of paired units.

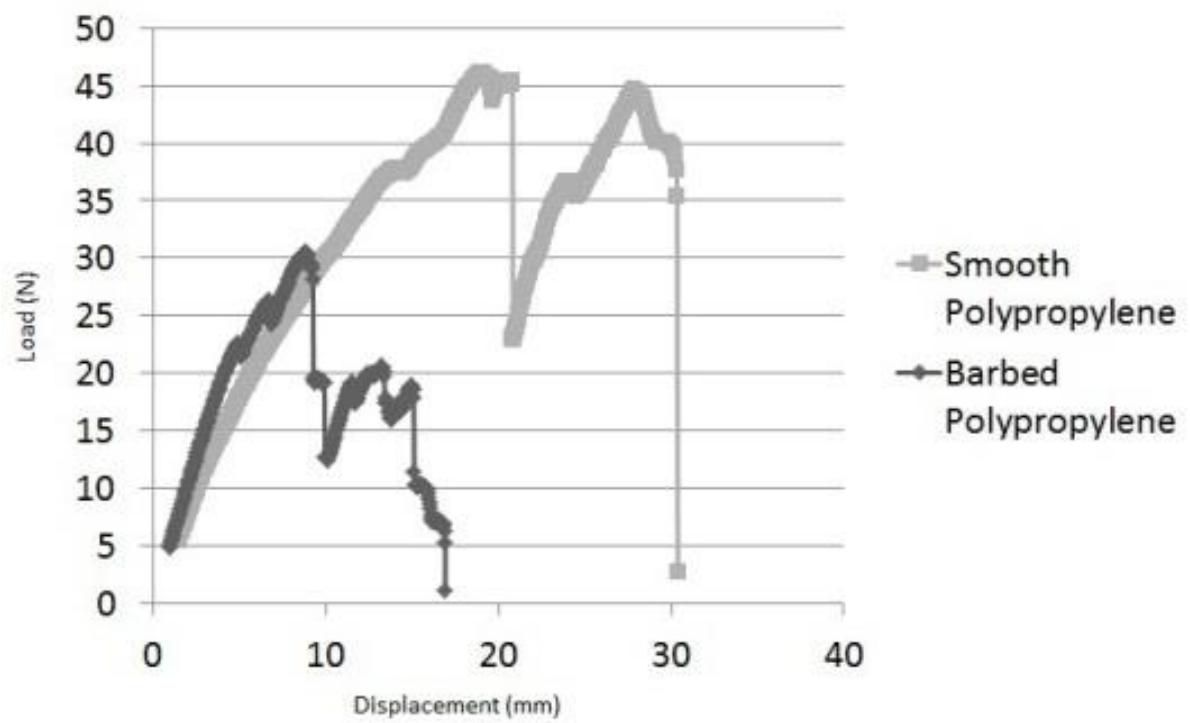
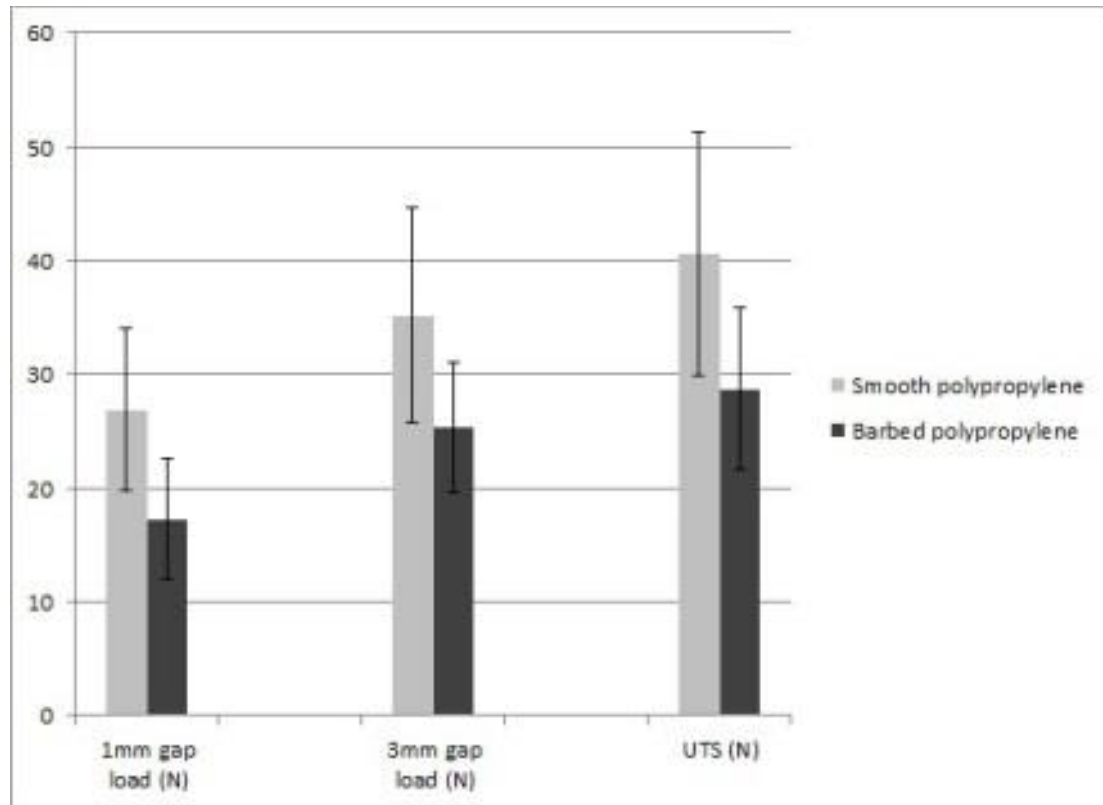


Figure 5. Results of servohydraulic tensile testing for load at 1 and 3 mm gap between cut surfaces and ultimate tensile strength (UTS).



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