Surgical repair of Achilles tendon rupture in dogs: a review of the literature, a case report and new perspectives

G. Spinella[1], R. Tamburro[2], G. Loprete[1], J.M. Vilar[3], S. Valentini[2]

[1]University of Catanzaro, Italy
[2]University of Bologna, Italy
[3]Universidad de Las Palmas de Gran Canaria, Spain

ABSTRACT: Achilles tendon rupture in the dog is a common traumatic lesion. An accurate evaluation of etiology, grade of the lesion with or without loss of tendon substance, and time elapsed from the trauma can guide the surgeon in choosing the best surgical technique. Moreover, the healing process after a tendon injury is usually difficult and uncertain because the prognosis is influenced by extent of trauma, time elapsed between the trauma and its treatment and low tendon vascularization. However, the rapid development of tissue engineering and regenerative medicine could soon result in the development of scaffolds with bioactive proteins that can direct the healing process toward complete tendon regeneration without fibrotic tissue development.

Keywords: *tendo calcaneus communis*; complete rupture; tissue ingeneering; polypropylene mesh; dog

List of abbreviations

TESF = transarticular external skeletal fixator; BMP = bone morphogenetic protein

Contents

1. Introduction

The Achilles tendon (*tendo calcaneus communis*) is the strongest tendon in the structure of the musculoskeletal system in the dog (Barone, 1981). In dogs the *tendo calcaneus communis* originates from different muscle-tendon units that conjoin at the heel: the gastrocnemius tendon, the superficial digital flexor tendon, and the common tendon of the biceps femoris, gracilis and semitendino-

sus muscles (Barone, 1981; Fahie, 2005; Lamb and Duvernois, 2005). Its main function is in rear-limb forward progression, and it contributes to passive support of the hock (Barone, 1981).

To reduce forces that affect the Achilles tendon with no negative impact on other areas, it is necessary to consider the three components of the "hock lever" system: the effort arm, the load arm and the amount of load (Robinson, 2007).The hock is a disadvantageous lever and even a small increase in

effort arm allows a significant reduction of forces applied on the Achilles tendon (Robinson, 2007). A greater length of effort arm should provide a decrease in the working arch of the hock with less work per step. Unfortunately, in the hock, the benefit obtained with this model would create overloads on other anatomical areas with associated posterior postural errors (Robinson, 2007).

The etiology of Achilles tendon injuries in dogs is usually traumatic. Depending on the trauma, the severity of the lesion may vary considerably, leading to stretching, small or partial lacerations or a complete rupture (Clark, 2001; Montgomery and Fitch, 2003). The nature of the trauma influences the type of tendon injury and may result in acute rupture of the tendon with partial or complete loss of integrity of the structure, sometimes with exposure of tendon ends (eg. hunting accident) (Clark, 2001). In addition, weakening or rupture of the tendon structure can occur secondarily to systemic diseases (e.g. Cushing's disease) or to iatrogenic etiologies (Montgomery and Fitch, 2003). Meutstege (1993) proposed classifying three different types of breakage, based on anatomical location and the severity of the lesion of the Achilles tendon: Type 1 is a complete rupture, Type 2 has three subtypes for partial rupture with a lengthened Achilles tendon system and Type 3 is a tendinosis or peritendinosis (Meutstege, 1993; Fahie, 2005).

The clinical signs associated with the rupture of the Achilles tendon will vary depending on the severity of the injury, but the "Plantigrade position" and the swelling around the tendon insertion on the hock are the main symptomes observed in a dog with complete rupture of the tendon.

The purpose of this article is to analyze the veterinary literature, focusing on surgical treatment and new surgical perspectives, presenting a particular clinical case of complete Achilles tendon rupture. For descriptive convenience, tendon surgical treatments will be divided into two sections: complete rupture of the tendon with no loss of substance and rupture with severe loss of substance.

2. Surgical treatment

2.1. Complete rupture with no loss of substance

The main objective of surgery for tendon injuries in the dog is to restore an adequate tensile strength

to support body weight (Fahie, 2005). Restoring gliding function for lower mobility and use of the phalanges, which is the primary objective of tendon surgery in humans, is instead a secondary objective in the dog (Fahie, 2005). Tendon vascularization requires precaution during surgery to minimize tissue trauma and to prevent excessive post-operative weight bearing (Fahie, 2005). The healing process of a tendon injury is usually difficult and uncertain because the prognosis varies widely depending on the amount of trauma and the time that elapses between trauma and treatment. Complicated recoveries are due mainly to:

(a) low vascularization of the tendon structure;
(b) a healing process that tends to create adhesions with surrounding tissues, restricting mobility;
(c) tension and active loads, which can provoke diastasis of tendon ends;
(d) active and passive tendon movements (Spadari, 2006).

In the preoperative period, tendon immobilization is necessary to prevent end separation (Spadari, 2006). In surgical repair, during acute injury, it is necessary to identify each singular component of the complex tendon, and these should be individually sutured. In the case of chronic lesions, however, the tendon ends are withdrawn, leaving a space filled by fibrous tissue. For this reason, identification of each component is not always possible. In this case, the fibrotic portion of the Achilles tendon is treated as a single structure (Fossum, 2004). Tendon rupture without loss of substance is treated with the patient under general anesthesia and in lateral recumbency. A limb hanging tension position provides an easier manipulation during surgery (Fossum, 2004). The skin incision should be parallel to the tendon so that skin healing does not complicate tendon repair. Subsequently, the tendon ends are identified, approached and sutured. The best results are achieved when the tendon ends are perfectly approached with a termino-terminal tenorraphy (Spadari, 2006). A non-absorbable suture, such as nylon or polypropylene, is preferable; however, steel wires, polidioxanone or polyglyconate may be also used. The recommended suture size ranges from 3 to 0 (USP scale) because the use of larger suture material could negatively affect the healing process by increasing tissue reaction. Also, the suture should not obstruct the local blood-supply and provoke diastasis of the tendon ends (Fossum, 2004).

The techniques for tendon anastomosis include different types of suture: Bunnel-Mayer, Mason-

Allen, simple interrupted, locking loop or modified Kessler, double locking loop, three-loop pulley, Krackow, continuous cruciate, and far-near-near-far (Fahie, 2005). Moores and colleagues compared the two locking loop suture with the three-loop pulley suture for the repair of canine Achilles tendons. The study showed that the three-loop pulley pattern is quicker to place and is more resistant to gap formation and to tensile load (Moores et al., 2004).

During post-operative management, primarily in the early stages of healing, limb immobilization is essential. Movement can produce a significant space between the tendon ends, decreasing the local blood supply and increasing fibrosis, which can compromise healing and final functional outcome (Clark, 2001). Immobilization techniques include transarticular external skeletal fixator (TESF), placement of a calcaneo-tibial bone screw, and various configurations of splints and casts (Guerin et al., 1998; Nielsen and Pluhar, 2006). Immobilization for six weeks allows restoration of about 50% of normal tendon strength but results in significant muscular weakness. In fact, joint fixation for more than three weeks could cause varus or valgus deformities. Fahie (2005) suggests complete post-op immobilization for three weeks, followed by a three weeks partial immobilization, allowing an acceptable restoration of function and reducing the risk of a new lesion.

Montgomery and Fitch observed that the strength obtained 6 weeks after tenorraphy is suitable for limited exercise, while the strength obtained after one year is appropriate for unlimited exercise (Montgomery and Fitch, 2003). Finally, Fossum (2004) suggests that after 3–6 weeks of fixation, the limb should be supported with a padded bandage to prevent dorsal tarsus flexion, and activity must be restricted to walking on a leash for 10 weeks. In a recent study, Nielsen and Pluhar (2006) analyzed healing and long-term results after surgical treatment, comparing different methods of tibio-tarsal immobilization in a group of 28 dogs that had partial (12 dogs) or complete rupture (16 dogs) of the Achilles tendon. Immobilization techniques used in the study were TESF (16 dogs), calcaneal tibial screw (one dog), and a splint or full cast (11 dogs). The study showed that there is no significant difference in recovery times between the use of TESF or bandage with splints; moreover, TESF application increased the duration of surgery (116 min versus 66 min) and costs due to more frequent patient

follow-ups (Nielsen and Pluhar, 2006). The study also showed that dogs treated within three weeks after trauma healed faster and with fewer complications. The average time of maintaining the system of immobilization was 10 weeks, while the average time of recovery for normal limb functionality was approximately 20 weeks (Nielsen and Pluhar, 2006).

2.2. Complete rupture with loss of tendon substance

Several studies have been published in veterinary literature on the treatment of rupture of the Achilles tendon in dogs, but few articles reported cases of rupture with massive loss of substance. The complete rupture of the tendon with loss of substance requires correct evaluation by the surgeon because of the impossibility of approaching tendon ends with a simple suture (Montgomery and Fitch, 2003). A particular surgical technique proposed by Demirkan et al. (2004) suggests the use of fascia lata grafts for the treatment of Achilles tendon rupture. The study was conducted in 20 New Zealand rabbits divided into two groups of 10 animals each. The first group received a fascia lata transplantation, while the second group received traditional surgery. Demirkan et al. (2004) observed that the fascia lata contributed significantly to the healing of the Achilles tendon, above all when a loss of tendon substance has occurred. Moreover, even if most of the grafts have rapid degeneration after implantation, the fascia lata graft persists for a longer period, induces fewer adhesions and is much more resistant to bacterial contamination.

Treatment of complete common calcaneal tendon rupture with loss of tendon length with a fascia lata graft was also successfully carried out by Shani and Sharar (2000). However, in human literature this technique is not advised because, in elderly patients (like in our clinical case), healthy tissue had to be sacrificed, which could produce a weakness of the limb (Choskey et al., 1996). This concern was also expressed by Shani and Sharar (2000). Swidersky et al. (2005) reported the successful use of fascia lata and polypropilene mesh to treat a bilateral partial rupture of the gastrocnemius tendon in a Labrador retriever.

Recently Maitre et al. (2009) described a case report of common calcaneal tendon recurrent, treated using the transposition of the caudal band

of the Sartorius muscle in addition to the usual tendon suture.

In 2007, Gilbert et al. conducted a clinical trial on the use of heterologous implants, obtained from porcine small intestine submucosa (Gilbert et al., 2007). During the experiment, 12 female dogs of mixed breeds and an average weight of 20 kg were divided into six groups, each composed of two animals each, and were subjected to experimental Achilles tendon rupture with a loss of 1.5 cm structure. For tendon reconstruction, a 2 × 3 cm natural implant was sutured using a 4-0 polypropylene suture and overlapping the tendon edges by about 2–3 mm (Gilbert et al., 2007). The use of submucosa implants, despite more rapid degradation compared with synthetic materials, allowed a quicker recovery. In fact, the dogs recovered almost full limb function soon after removal of the bandage. Moreover, the carbon ^{14}C residue showed no contamination of other tissues from the scaffold degradation products, which were removed only by urine. The authors concluded that the use of porcine small intestinal submucosa implants were a valid and effective alternative for reconstruction of the Achilles tendon because they provided quick healing and deposition of new tissue, thanks to chemotactic peptides released during the degradation of the implant. However, due to rapid degeneration, post-operative immobilization and exercise limitation were imperative in the first few weeks after surgery (Gilbert et al., 2007).

The polypropylene mesh is used widely in surgery, especially in orthopaedic reconstructive surgery, as a synthetic implant (Ozaky et al., 1986; Hosey et al., 1991;Choskey et al., 1996; Swiderski et al., 2005). It is chemically inert, hypoallergenic, and capable of withstanding mechanical stress; in addition, its porosity allows fibrous tissue ingrowth (Swiderski et al., 2005). In 1991 Hosey and co-workers, in an experimental study on rabbits, demonstrated that the polypropylene mesh acted very closely to the physical properties of a normal Achilles tendon (Hosey et al., 1991). Moreover, they proved that this material provided a frame or bridgework for ingrowth collagen bundles (Hosey et al., 1991). In 1996, Choskey et al. described the repair of a neglected Achilles tendon in five human patients producing satisfactory results using, for tendon reconstruction, a tunnel technique with a Marlex mesh (Choskey et al., 1996). On the basis of these reports, we focused our attention on the use of polypropilene mesh alone as a method for tendon repair in the following clinical case.

3. Case report

A 12-year-old female English Setter dog was referred to the veterinary clinic for severe lameness due to trauma that occurred a few hours before during a hunting expedition. Physical examination showed an open wound on the right leg region. The trauma involved the skin, subcoutaneous tissue and the Achilles tendon. The tendon appeared completely interrupted with a severe loss of tendon structure, resulting in *non-weight bearing lameness*. X-ray examination in the medio-lateral view showed a calcaneal bone infraction with no evidence of avulsion tendon injury. Standard preoperative bloodworks was in the normal range.

The dog was anesthetized and positioned in left lateral recumbency. A surgical debridement of the wound was performed, and the necrotic tissue was removed. Tendon ends were isolated from the surrounding tissue, and the margins were debrided, irrigated and modelled in order to become regular. The apposition of tendon ends was not possible either in a physiological position or in maximum extension of the hind limb; in the latter position, the tendon fault was 2 cm. A polypropylene mesh (Prolene, Ethicon, Somerville, NJ, USA) was used to create an anatomical no-tension reconstruction. The mesh was folded in two layers and rolled in order to obtain a cylinder 7 cm in length and 1 cm in diameter. Tendon ends were located entirely into the mesh with a tunnel technique, creating a bridge between the two ends (Figure 1). An interrupted synthetic non-absorbable monofilament suture (2-0 Nylon) was applied between the mesh and the tendon with a distance of about 3 mm between the single knots. This procedure was performed maintaining the hock joint in a mild extension (about 130° angle). Skin was closed with a no-tension interrupted suture.

A bandage (in physiological position) with a craniocaudal splint was necessary for 10 weeks, both to protect the limb from infections and to support and immobilize the tibiotarsal joint. After 10 weeks, the dog was re-evaluated and was found able to bear partial weight. On palpation, the tendon tract appeared swollen, irregular and firm. The splint was removed, and a simple bandage was applied for another month. The owner was informed
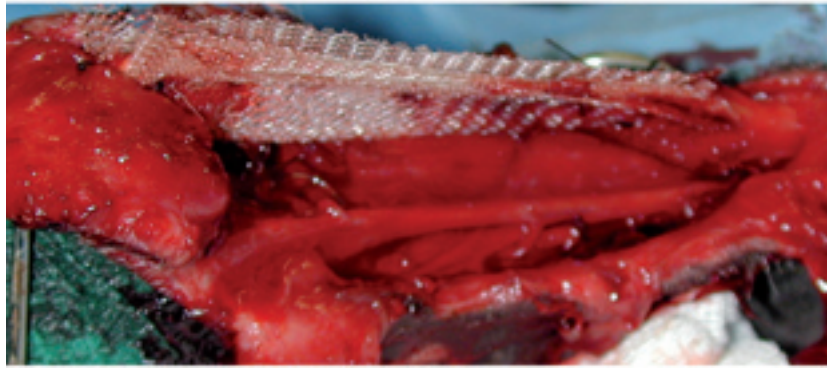
Figure 1. Twelve-year-old female English Setter dog. Marlex mesh has been used in order to create a bridge between the two ends of the Achilles tendon

to restrict the dog's activity and to perform passive physiotherapy by softly extending and flexing the joint to prevent tendon adhesion and muscular hypotrophy.

An appropriate anti-inflammatory treatment (Carprofen, 2 mg/kg, bid, *p.o.*) was prescribed for the first 15 days, while antibiotic treatment (Clavulanic acid and Amoxicillin trihydrate, 12.5 mg/kg, bid, *p.o.*) was prolonged for one month in order to avoid postoperative wound infection.

An ultrasonographic follow-up of the tendon was done every two weeks up until two months and at eight months postoperative. A complete recovery with bearing of weight and no lameness or swelling in the surgical site was observed after eight months (Figure 2). No biopsy was performed, but the ultrasonographic examination and palpation showed a structure with an echogenicity and a consistency very close to the tendon structure. In our experience,

the polypropylene mesh was relatively simple to apply and required no special equipment. Moreover, no healthy tissue was removed and no invasive external fixation was required to immobilize the tibiotarsal joint, reducing surgical invasiveness.

In our clinical case, immobilization was obtained using a bandage with a craniocaudal splint with no complications. The recovery time of our case was longer than the mean time reported by Nielsen; however, in that article cases were reported which required 12 months for a complete recovery (Nielsen et al., 2006). Therefore, we have assumed our patient's recovery time as acceptable, considering patient age, the good functional outcome and the particular surgical technique.

This description, although it reports only a single case, confirms the efficacy of polypropylene mesh for tendon reconstruction in the dog. As suggested by other authors, it is important to note that limb

Figure 2. Eight months after surgery. Hock joints have almost the same range from the ground

physiotherapy in the postoperative period is essential to prevent tendon adhesion and a muscular atrophy (Shani and Sharar, 2000; Montgomery and Fitch, 2003; Swiderski et al., 2005).

4. New perspectives

The regeneration of tendon tissue is the main objective of tissue engineering and regenerative medicine. Great improvements have been made in the regeneration of skin tissue, as well as in regeneration of bone and ligaments. However, recent studies have revealed a new approach for the treatment of tendon and ligament injuries through the use of scaffolds in synthetic material. The objective of research in tissue engineering and regenerative medicine is to design and create a reproducible, bioactive and bioresorbable 3-dimensional scaffold with tailored properties that are able to maintain their integrity for predictable times, even under load-bearing conditions.

Guarino et al. (2007), in a recent review analyzed different approaches to utilizing bioactive scaffolds for regeneration of damaged tissue in bones and ligaments. They propose that scaffolds be improved with the incorporation of insoluble and soluble signals capable of promote cellular activities aimed at tissue regeneration (Guarino et al., 2007).

Two different strategies may be taken into consideration for tissue regeneration: the aforementioned regenerative medicine and tissue engineering. These two fields of research require specific cell isolation through a small biopsy from the patient and cell growth on the scaffold. The strategy of regenerative medicine is to implant the scaffold directly into the patient, with the aim of stimulating new tissue formation. On the other hand, tissue engineering requires two steps: a first step of modulation of the scaffold components *in vitro*, and a second step of implantation. The scaffolds are conditioned by bioreactors, which are capable of regulating all specific physicochemical cultural parameters (pH, temperature, pressure, etc.) required to reproduce an ideal environment for *in vitro* tissue development. In both approaches, a critical aspect is the development of a scaffold that acts as a temporary matrix for cell proliferation and provides deposition of an extracellular matrix (Guarino et al., 2007).

As a first step in a structural properties study, it will be necessary to identify a mechanism of cell adhesion, proliferation and differentiation, which simulates the structural complexity of natural tissue. Subsequently, critical aspects of the scaffold are porosity, interconnectivity between pores and their sizes in order to ensure uniform distribution of cells, their survival, proliferation and migration and to promote neo-vascularization. Neoangiogenesis is the critical point in providing adequate nutrient transportation and toxic metabolite removal (Guarino et al., 2007). For bone, a pore size of 150 μm has been suggested, while the size for ligament and tendon has been suggested to be 200–250 μm (Guarino et al., 2007).

Another fundamental aspect for the design of a scaffold is the choice of a suitable material. Depending on the origin (synthetic or natural) and nature (organic and inorganic) of the material, it is possible to distinguish four different characteristics of biomaterial for use in the construction of an implant (Guarino et al., 2007): the ideal scaffold for ligament and tendon tissue should be biodegradable, biocompatible and porous, have sufficient mechanical strength and promote the formation of new tissue (Guarino et al., 2007). Ideal scaffold materials for ligament and tendon are collagen, silk and biodegradable polymers. However, the main obstacle to these compounds is their architecture. To overcome this limitation, a matrix composed of silk fibres has recently been developed that simulates the arrangement of collagen fibres in natural ligaments/tendons. These scaffolds with a three-dimensional matrix are able to better adapt to the implant sites and stimulate the growth and development of new tissue (Guarino et al., 2007). Bioactivity is the main strategy in the modern development of biomaterials, which addresses the appropriate response to external stimuli. The objective is to develop a new type of material that stimulates a biochemical response from living tissue to give a strong biological link between the implant and the tissue. In this context, the use of materials with slow degradation allows maintenance of bioactive components of scaffolds *in situ* long enough to drive tissue growth and provide for its maturation (Guarino et al., 2007).

Bioactive molecules include bioactive proteins and growth factors (bone morphogenetic proteins [BMPs], growth factor β, insulin growth factor, fibroblast growth factor). In addition, an alternative approach involves the use of oligosaccharides and oligopeptides. Cell adhesion to traditional biomaterials is based on recognition by proteins such

as fibronectin, fibrinogen and vitronectin, which are absorbed in a non-specific way and promote cell adhesion through interaction with the corresponding receptors. The advantage of using small peptides rather than whole proteins is the lower cost and greater manoeuvrability (Guarino et al., 2007). Of particular interest is the study conducted by Forslund (2003) on rats and rabbits regarding the use of BMPs for the treatment of experimental rupture of the Achilles tendon. The study included the evaluation of the use of BMPs in the healing process of Achilles tendon rupture, the identification of their appropriate concentration and an effective method of administration to prevent associated side effects, such as bone or cartilage generation into the tendon. In the experiments, three types of BMPs were used: BMP-12, BMP-13, BMP-14. From the results obtained, it was observed that the BMP-13 are the most effective because they are more effective during the differentiation of mesenchymal cells in connective tissue than the other two types, which can cause the formation of bone or cartilage islands (Guarino et al., 2007).

These last concerns lead us to conclude that in the future, close interaction between veterinary surgeons and biomedical engineers may promote the creation of tendon scaffolds with specific BMPs that can speed up the healing process and total tissue regeneration.

5. REFERENCES

Barone R (1981): Comparative Anatomy in Domestic Animals (in Italian and French). Vol. 2. Edagricole, Bologna. 790–791.

Choskey A, Soonawalla D, Murray J (1996): Repair of neglected Achilles tendon ruptures with Marlex mesh. Injury 27, 215–217.

Clark DM (2001): Tendon lesion and its treatment. In: Bojrab M J (ed.): Disease Mechanisms in Small Animal. Giraldi, Bologna. 1414–1418.

Demirkan I, Atalan G, Cihan M, Sozmen M, Kankavi O (2004): Replacement of ruptured Achilles tendon by fascia lata grafting. Veteriner Cerrahi Dergisi 10, 21–26.

Fahie MA (2005): Healing, diagnosis, repair, and rehabilitation of tendon conditions. Veterinary Clinics Small Animal Practice 35, 1195–1211.

Forslund C (2003): BMP treatment for improving tendon repair: Studies on rat and rabbit Achilles tendons. Acta Orthopaedica Scandinavica, 74 (Suppl. 308), 1–30.

Fossum TW (ed.) (2004): Small Animal Surgery. 2nd ed. Elsevier, Milano. 1150–1159.

Gilbert T W, Stewart-Akers AM, Simmons-Byrd A, Badylak SF (2007): Degradation and remodeling of small intestinal submucosa in canine Achilles tendon repair. Journal of Bone & Joint Surgery 89, 621–630.

Guarino V, Causa F, Ambrosio L (2007): Bioactive scaffold for bone and legament tissue. Expert Review of Medical Devices 4, 405–418.

Guerin S, Burbridge H, Firth E, Fox S (1998): Achilles tenorrhaphy in five dogs: a modified surgical technique and evaluation of a cranial half cast. Veterinary and Comparative Orthopaedics and Traumatology 11, 205–210.

Hosey G, Kowalchick E, Tesoro D, Balazsy J, Klocek J, Pederson B (1991): Comparison of mechanical and histologic properties of Achilles tendons in New Zealand white rabbits secondarily repaired with Marlex mesh. Journal of Foot Surgery 30, 214–233.

Lamb CR, Duvernois A (2005): Ultrasonographic anatomy of the normal canine calcaneal tendon. Veterinary Radiology & Ultrasound 46, 326–330.

Maitre P, Carozzo C, Genevois JP, Fav D, Viguier E (2009): Trasposition of Sartorius muscle for treatment of recurrent common calcaneal tendon rupture. In: Proceedings of 18th Annual Scientific Metting, Nantes, 371–374.

Meutstege FJ (1993): The classification of canine Achille's tendon lesions. Veterinary and Comparative Orthopaedics and Traumatology 6, 53–55.

Montgomery R, Fitch R (2003): Muscle and tendon disorders. In: Slatter D (ed): Textbook of Small Animal Surgery. 3rd ed. Saunders, Philadelphia. 2266–2267.

Moores AP, Owen MR, Tarlton JF (2004): The three-loop pulley suture versus two locking-loop sutures for the repair of canine Achilles tendons. Veterinary Surgery 33, 131–137.

Nielsen C, Pluhar GE (2006): Outcome following surgical repair of Achilles tendon rupture and comparison between post-operative tibiotarsal immobilization methods in dogs. Veterinary and Comparative Orthopaedics and Traumatology 19, 246–249.

Ozaky J, Fujimoto S, Masuhara K, Tamai S, Yoshimoto S (1986): Reconstruction of chronic massive rotator cuff tears with synthetic materials. Clinical Orthopaedics 202, 173.

Robinson S (2007): Understanding the Hock. In: "Darkstarr Rottweilers" online website http://www.dkstarr.com/hock.thm.

Shani J, Sharar R (2000): Repair of chronic complete traumatic rupture of the common calcaneal tendon in a dog, using a fascia lata graft – case report and lit-

erature review. Veterinary and Comparative Ortho-
paedics and Traumatology 13, 104–108.

Spadari A (2006): Notes on Veterinary Surgery (in Italian).
2nd ed. Societa Editrice Esculapio, Bologna. 233–242.

Swiderski J, Fitch RB, Staaz A, Lowery J (2005): Sono-
graphic assisted diagnosis and treatment of bilateral
gastrocnemius tendon rupture in a Labrador retriever
repaired with fascia lata and polyprolylene mesh. Vet-
erinary and Comparative Orthopaedics and Trauma-
tology 18, 258–263.

Corresponding Author:

Dr. Giuseppe Spinella, University of Catanzaro, Experimental and Clinical Department, Campus "S.Venuta", Viale
Europa, 88100 Germaneto (CZ), Italy

Tel. +39 961 3694232, Fax +39 961 3695031, E-mail: spinella@unicz.it