Fracture repair
The use of ultrasound in bone healing

Feline hypertension
Comparing healthy cats to those with CKD

Outcomes of GDV
Prognostic value of blood parameters

Companion animal rehabilitation

Also in this volume:
Exercises in physical rehabilitation, prognostic role of pulmonary hypertension in MMVD dogs, the role of the 'tongue worm'.

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Reprint paper*

Physiotherapy in small animal medicine

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ABSTRACT

The benefits of physiotherapy have been extensively demonstrated in human medicine. Although physiotherapy has been performed in veterinary medicine for several decades, it is only very recently that scientific research on this subject is increasing. The purpose of this paper is to give an overview of the different veterinary physiotherapeutic assessment and treatment techniques and possibilities, and correlate them to the data in the veterinary literature.

Introduction

The benefits of physiotherapy in human medicine have been known for many decades and nowadays, it has been incorporated in the plan of care of conditions, like cruciate ligament rupture (Anderson and Lipscomb, 1989; Shelbourne and Nitz, 1990; Shelbourne et al., 1991), fracture repair (Kristiansen et al., 1997; Sherrington and Lord, 1997; Diong et al., 2015), joint arthroplasty (Moffet et al., 2004; Denis et al., 2006), spinal surgery (Ostelo et al., 2003; Ostelo et al., 2009) osteoarthritis (OA) (Dias et al., 2003), lower back pain (Aure et al., 2003) and many other conditions (Levine et al., 2005). Although physiotherapy has been used over twenty-five years in veterinary medicine (Prydie and Hewitt, 2015) and several studies have been published on how to perform animal physiotherapy (Bockstahler et al., 2004; Zink and Van Dyke, 2013; Millis and Levine, 2014), in the veterinary literature, physiotherapy is poorly documented.

The goal of this paper is to review the veterinary physio-therapeutic possibilities and to explore what is known on the effect of animal physiotherapy in the literature.

The goals of physiotherapy

Independent of the species, the goals of physiotherapy are always the same (Levine et al., 2005; Sharp, 2008): reduce pain, facilitate healing, increase (or maintain) muscle strength, restore normal osteokinematic and artrokinematic movement of joints, increase the general condition and restore normal functionality.

Based on the findings on physiotherapeutic assessment (symmetry, kinematics, end feel, flexibility, translation, etc.), the physiotherapist can choose out of three therapeutic categories to achieve these goals (Prydie and Hewitt, 2015). They can either be used separately or in combination. The first category is manual therapy. This therapy includes all techniques that involve the use of the hands of the physiotherapist. It is mainly used to reduce pain and help with loss of motion secondary to neuromusculoskeletal disorders (Saunders et al., 2005). The second category is the use of therapeutic modalities. It involves every technique, in which an external energy source is used to stimulate or support the healing process (Prydie and Hewitt, 2015). The third category consists of therapeutic exercises. This category holds every exercise that actively trains the muscles and improves the mobility of the joints. These exercises also help in training coordination, proprioception and core stability. In a later stage, they are indispensable to train muscles and improve cardiorespiratory fitness. (Prydie and Hewitt, 2015).

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A lot of these exercises are part of the home exercise program. Owners should continue to revalidate/ train their pet at home using the guidelines given by the physiotherapist. As it is a very important part in physiotherapy, good instructions on how to perform the exercises are required (Prydie and Hewitt, 2015).

Manual therapy

Manual therapy is a term that covers all soft tissue techniques used in (animal) physiotherapy with the intention to soothe pain, improve tissue extensibility, increase range of motion (ROM), change muscle tension (relax or stimulate), manipulate soft tissue and joints, reduce swelling and inflammation and improve the general circulation (Zink and Van Dyke, 2013). Manual therapy principally consists of soft tissue mobilization (i.e. massage), joint mobilization and passive movement (pROM) (i.e. cycling movements). Although the techniques of different types of manual therapy are well described in the veterinary literature, to date, only limited information can be found regarding clinical results in animals (Bockstahler et al., 2004; Saunders et al., 2005; Zink and Van Dyke, 2013; Prydie and Hewitt, 2015). Most references are based on data found in the human literature and provide controversial and limited documentation on soft tissue mobilization (Hertling and Kessler, 2006; Zink and Van Dyke, 2013). Studies on joint mobilization and pROM show positive effects in humans, but research data are lacking in veterinary medicine (Landrum et al., 2008; Zusman, 2010). Although the principles are similar, straightforward interspecies extrapolation should be done with caution. In one study, massage was actively incorporated in the physiotherapeutic protocol of dogs with degenerative myelopathy, besides active and passive exercises and hydrotherapy (Kathmann et al., 2006). This report demonstrated that intensive physiotherapeutic treatment may prolong life expectancy by a factor five compared to dogs without physiotherapy. However, the study design could have biased this result, as the owners were involved in the group selection.

Therapeutic modalities

Therapeutic modalities use physical forces, such as temperature, electric current, sound and light to create an effect on tissue. Each of these modalities is discussed below.

Temperature

The use of temperature changes is one of the oldest forms of physiotherapy and is easily accessible to veterinarians and owners (Olson and Stravino, 1972; Millis and Levine, 2014). The purpose of heat and cold treatment is to decrease pain, reduce swelling, improve flexibility and promote overall healing. The principle is based on universal physiologic cell reactions. Cold induces vasoconstriction decreases blood flow, muscle spasm and tissue swelling, reduces metabolism and enzyme-mediated tissue damage and provides analgesia by decreased nerve conduction velocity. Heat has the opposite effect. It induces vasodilatation and leukocyte migration, increases the blood flow, soft tissue extensibility and metabolism, relaxes muscles and relieves pain (Michlovitz, 1996; Millis and Levine, 2014).

Cold

A study by Bocobo et al. (1991) investigated the optimal application of cryotherapy in the dog. Icepacks were used on the stifle joint for 5, 15 or 30 minutes and intra-articular as well as rectal core temperatures were noted. A linear drop of intra-articular temperature was noted with a longer period of cooling. The core temperature was minimally affected up to 15 minutes of treatment (0.1°C). Thirty minutes of cooling resulted in a further 0.5°C drop of core temperature. The effect of cooling remained for another 21.7 to 33.2 minutes. The use of ice water emersion resulted in a much higher temperature drop both in intra-articular and in core temperature, and in a longer lingering effect of approximately one hour (Bocobo et al., 1991). Wakim et al. (1951) found that using icepacks on the canine stifle for more than 30 minutes does not cause additional effect. Therefore, it can be concluded that the optimal duration of local cryotherapy on the canine stifle, with minimal effect on the core temperature, is 15 to 30 minutes, with an ideal of 20 minutes (Millard et al., 2013). For optimal effect, this treatment is to be repeated two to four times a day (Millis, 2004).

The effect of cryotherapy on the stifle has been investigated in two studies. In one study, it could be demonstrated that postoperative tissue swelling after extracapsular stifle surgery decreased significantly with the use of icepacks (all or not combined with bandaging), compared to bandaging alone (Rexing et al., 2010). In another study, the effects of

cryotherapy after tibial plateau levelling osteotomy (TPLO) were described. A significant lower pain score, lower lameness score, less swelling and better ROM in the first 24 hours after surgery were demonstrated (Drygas et al., 2011).

Heat

The easiest way to apply heat to the body is to use superficial agents such as hot packs (Millard et al., 2013; Millis and Levine, 2014). Heat can either be used on soft tissues that entered the healing phase (48 hours post trauma at the earliest) or in cases of chronic pain (Millis, 2004; Millard et al., 2013). However, no scientific data on the actual healing effect of heat in small animal medicine can be found to date (Millard et al., 2013). Besides the purely healing effect, heating soft tissue also has another function. When applying heat prior to stretching and exercise, it might cause less tissue damage and a larger ROM (Millis, 2004; Millis and Levine, 2014), facilitating other physiotherapeutic exercises. A recent study on the heating effect of warm compresses on the lumbar region in dogs demonstrated that a 10-minutes application of a 47°C compress resulted in a 4.14° increase at 0.5 cm depth, 2.2° increase at 1cm depth and 0.58° at 1.5 cm depth. Core temperature was not affected. Shorter application resulted in lower increase of temperature; longer application did not show significant increase of temperature. Studies on the duration of the heating effect are not available to date (Millard et al., 2013).

To heat deeper structures up to 5 cm, external heat compresses are not sufficient. Other modalities such as continuous ultrasonography or infrared/laser should be considered (Millis, 2004; Steiss and Levine, 2005).

Electric current

Electrical stimulation in small animal physiotherapy has mainly been used with the intention to ease pain or to stimulate muscle and/or nerve function (Bockstahler et al., 2004; Steiss and Levine, 2005; Zink and Van Dyke, 2013; Prydie and Hewitt, 2015). In veterinary medicine, devices used for electrical stimulation are generally small portable units powered by a nine-volt battery. The device has either one or two power cords leading to the electrodes (Figure 1). The better-equipped devices both have a pain reduction and muscle stimulating function.

Figure 1. Neurotrac® TENS/NMES device.

Transcutaneous Electrical Nerve Stimulation (TENS)

This is a type of electrical stimulation especially used for pain control (Bockstahler et al., 2004). The principle of TENS is based on the Gate Control Theory, that proposes a mechanism in the dorsal horns of the spinal cord that acts like a gate that can either inhibit or facilitate transmission from the body to the brain on the basis of the diameters of the active peripheral fibres, as well as the dynamic action of brain processes (Melzack and Wall, 1965) (Figure 2).
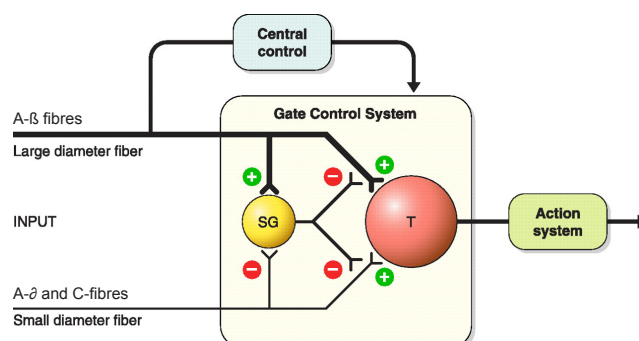
Figure 2. Gate control theory (Adapted from Zeilhofer et al., 2012)

Neural systems have three important types of fibres (Bockstahler et al., 2004):
- Aβ fibres are fast transmitting fibres for vibration and pressure sensation.
- Aδ and C fibres are slow transmitting fibres conducting pain signals.
- Substantia gelatinosa (SG) cells inhibit the pain signal to the brain.

By over-stimulating the Aδ and C fibres, SG cells get activated and the pain signal is blocked. In other words, the brain receives an overload of information, resulting in a blocked transmission of pain signals (Fox, 2013).

TENS has been demonstrated efficient in orthopaedic and neurologic conditions in humans, but research in the veterinary field is still limited (Millis and Levine, 2014). In one study, the effect of TENS on five osteoarthritic canine stifle joints was evaluated using force plate evaluation. A significantly improved weight bearing could be demonstrated, starting from 30 minutes until 210 minutes after treatment, with a peak improvement at 30 minutes (Levine and Millis, 2002).

Mlacnik et al. (2006) investigated the possible benefit of TENS combined with a weight loss program in dogs with several types of lameness. It was observed that dogs that had TENS not only lost weight faster than dogs without TENS, but also showed better weight bearing (validated by force plate) than the other group (Mlacnik et al., 2006).

Neuro-muscular electrical stimulation (NMES)
With neuro-muscular electrical stimulation (NMES), an electrical current is used to stimulate a muscle or nerve using an intact nerve (Bockstahler et al., 2004). Giving an electrical impulse to the neuromuscular unit results in an initial contraction of the faster type II muscle fibres, followed by contraction of the slower type I muscle fibres (Figure 3). The power of an NMES induced muscle contraction is lower than that of a voluntary muscle contraction, but often, a maximal voluntary muscle contraction is impossible or undesired after injury or surgery. In those cases, NMES can help to maintain or revalidate the muscle function (Steiss and Levine, 2005; Millis and Levine, 2014).

Figure 3. The use of NMES on the front leg of a dog.
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NMES has been used in a series of orthopaedic and neurologic conditions in humans and has become standard plan of care in many conditions (Millis and Levine, 2014). Data in the veterinary literature are scarce but promising.

In an experimental study in dogs, Johnson et al. (1997) demonstrated a significantly greater thigh circumference, an improved subjective lameness score and a lower degree of osteoarthritis. Millis and Levine (2014) investigated the difference in revalidation between ten dogs that had lateral suture surgery for cranial cruciate ligament (CCL) rupture. Five of the dogs received standard postoperative care consisting of rest and leash walks while the other five received additional ROM and walking exercises combined with NMES. A significantly greater thigh circumference and ROM were noted in the exercise group than in the group of dogs that received standard postoperative care (Millis and Levine, 2014).

Because electrical pulses are generated to pass through tissues, animals with cardiac problems or pace makers should not receive NMES treatment. The therapy is also contraindicated in animals with a history of seizures.

Sound waves

Therapeutic ultrasound
In therapeutic ultrasound (US), energy created by vibration of a piezoelectric crystal is used. Due to an electrical current, the crystal starts to vibrate and creates ultrasonic sound waves (Figure 4). The frequency of the waves depends on the electrical current sent through the crystal. The amount of energy or intensity carried by the sound wave corresponds with the amplitude and is usually measured in Watt per cm² (Bockstahler et al., 2004).
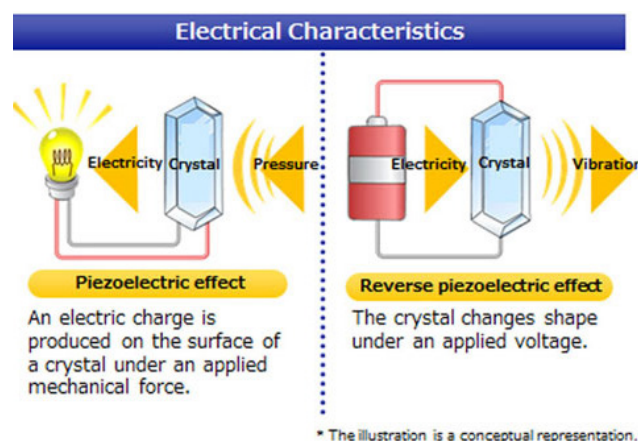

Figure 4. Mechanism of a piezoelectric crystal (Source: Global Epson).

Sound waves migrate easily in an aquatic environment and are attenuated in an air environment. Therefore, hair should be clipped and a water-based medium, such as water-soluble ultrasound gel, should be used to

Figure 5. Ultrasound treatment of a hip joint in a dog. © Dept of Medical Imaging and Small Animal Orthopaedics, Ghent University

accommodate the energy transfer (Millis and Levine, 2014) (Figure 5). Energy absorption is higher in tissues with high protein content. In terms of tissue types, this results in (low to high absorption) blood, fat, muscle, blood vessels, skin, tendon, cartilage and bone.

The effect on tissues depends on the type of ultrasound that is used: continuous wave (CW) ultrasound generates a continuous stream of energy, resulting in a tissue heating effect. In pulsed wave (PW) ultrasound, the energy stream is intermittently on and off. The latter mode is used when non-thermal effects are desired (Bockstahler et al., 2004; Prydie and Hewitt, 2015).

Continuous wave (CW)

This application was the initial goal of ultrasonographic therapy. Tissue heating is claimed to have an influence on a variety of conditions such as increased collagen extensibility, blood flow, nerve conduction, enzyme activity and decrease in pain sensation. The depth of the heating effect is determined by frequency and amplitude. Most devices have 1MHz- and 3MHz-frequency settings. 1MHz frequency delivers heat at a depth of 2 to 5 cm, 3MHz frequency between 0.5 and 3 cm of depth. The higher the amplitude, the more energy is delivered, the higher and faster the temperature increases (Millis and Levine, 2014).

In a study with ten dogs, a 3MHz probe was used to induce an increase of temperature in the thigh muscles. The difference in temperature was measured using heat sensitive needles at 1, 2 and 3cm of depth. Depending on the intensity, an increase up to 4.6°C, 3.6°C and 2.4°C, respectively, could be demonstrated. This effect remained present for about ten minutes (Levine et al., 2001).

It can be concluded that CW ultrasound is useful for heating tissue to at least 3 cm of depth and that an increase of amplitude results in an increase of tissue temperature.

Pulsed wave (PW)

Contrary to CW, the aim of PW is not to heat the tissue but to deliver energy to the deeper tissues. This energy can be used to repair either soft tissue or bone. Looman et al. (2003) demonstrated that PW does not have a thermal effect. In their US study on tendon tissue, they found that the thermal effect of PW is significantly lower (increase of temperature lower than 1.5°C) than in case of CW. Contrary to what is seen in the CW, the increase of intensity gave no significant increase in the temperature of the tendon tissue (Loonam et al., 2003).

Pulsed wave ultrasonography aids in the modulation of the inflammatory process (Millis and Levine, 2014). By stimulating the platelets, neutrophils, macrophages and causing mast cells to degranulate, the inflammatory cascade is facilitated (Maxwell, 1992). Therefore, the inflammation period should run smoother and faster.

In human medicine, PW is used for several soft tissue conditions, with good result. The main indications in humans are tendinitis, bursitis, joint contracture, pain, muscle spasms and treating of scar tissue (Millis and Levine, 2014). Reports on soft tissue repair are scarce in the veterinary literature. There is one study on rats and one on rabbits investigating muscle trauma and ear trauma, respectively, but without any hard evidence of effects on long term. In the acute phase, PW is said to be beneficial (Dyson et al., 1968; Rantanen et al., 1999). Although it is

most likely that animals also benefit from PW, it has yet to be proven.

More research has been performed on bone repair. Several studies in rats and dogs have demonstrated a beneficial effect of PW in the acute phase of bone healing, and even in the process of delayed- and non-union (Zorlu et al., 1998; Rantanen et al., 1999; Tanzer et al., 2001; Rawool et al., 2003; Rodrigues et al., 2004; Schortinghuis et al., 2004; de Sousa et al., 2008; Favaro-Pipi et al., 2010; Mosselmans, 2011; Mosselmans et al., 2013; Toy et al., 2014).

Shockwave

Shockwave therapy is based on the creation of high pressure and high velocity sound waves that are sent through the skin to the desired location. Based on the density of the tissue, more or less energy is released. The main differences between ultrasound and shockwave therapy is that the latter does not induce heat, has a lower frequency and minimal tissue absorption (Millis and Levine, 2014).

The limited studies that are available in small animal veterinary medicine focus on pain relieve in dogs with osteoarthritis (OA) of the hip (Mueller et al., 2007), OA of the stifle (Dahlberg et al., 2005) and dogs with patellar desmitis post-TPLO (Gallagher et al., 2012). In all of the studies, a positive effect of shock wave therapy was reported, although significant changes were only noted in the studies on hip OA and patellar desmitis. The positive effect of shockwave therapy on OA in elbows was demonstrated in fifteen dogs (Millis et al., 2011).

The effect of shockwave therapy on soft tissues, tendons, ligaments and wound healing has mostly been examined in laboratory animals, serving as a human model. Positive effect has been demonstrated, but up till now, data on dogs are lacking. Preliminary results on the effect of bone healing post-TPLO are promising but need to be further investigated on fractures or delayed union (Kieves et al., 2015).

Because of the intensity of the waves, shockwave is not indicated in case of neoplasia, acute inflammation, recent surgery, presence of implants, unstable fractures, neurologic deficits, immature animals and coagulation disorders (Bockstahler et al., 2004; Millis and Levine, 2014).

Although promising effects were demonstrated on OA, wound healing, fracture and ligament healing, more research in the canine field is necessary to estimate the real value of shockwave therapy.

Light Therapy

For many centuries, healing effects have been attributed to light. Over the last years, laser (i.e. light amplification by stimulated emission radiation) therapy has become increasingly popular for the treatment of a variety of conditions. For physiotherapeutic purposes, there are two main groups: cold lasers or low-level laser therapy (LLLT) and therapeutic lasers (Millis and Levine, 2014). The classification of the laser devices is based on their power. LLLT lasers are classified as Class 3 and have a wavelength up to 500 milliwatts (mW), while the more powerful therapeutic lasers are classified as Class 4 (higher than 500 mW) (Prydie and Hewitt, 2015). A more detailed description on the classification of lasers is not subject of this review and can be found on the website of the American National Standards Institute (ANSI).

The effect of laser is based on the emission of different wavelengths that are absorbed by the chromophores in different types of tissues. Each tissue has a different concentration of chromophores and the wavelength of the laser influences the absorption by the chromophores (Millis and Levine, 2014). The physiotherapeutic effect of laser therapy may vary, depending on the type of tissue and the different wavelengths of the laser.

Wavelengths under 600 nanometre (nm) are mostly absorbed and scattered by melanin and haemoglobin, and thus have little biologic effect. Wavelengths over 1400 nm are absorbed by water, and again have no biologic effect. Therefore, the optimal wavelength for therapeutic lasers should be between 600 and 1200 nm (Figure 6).

The wavelength also influences the depth of penetration. Longer wavelengths penetrate deeper (up to 2 cm direct penetration and 5 cm indirect penetration) than shorter wavelengths. Therefore, lasers with shorter wavelengths may be used for superficial injuries, while lasers with a longer wavelength may work deeper. Studies on skin penetration have mainly been performed on human skin (Kolarova et al., 1999; Esnouf et al., 2007). The effect of the skin composition and coat of animals on the penetration is currently unknown.
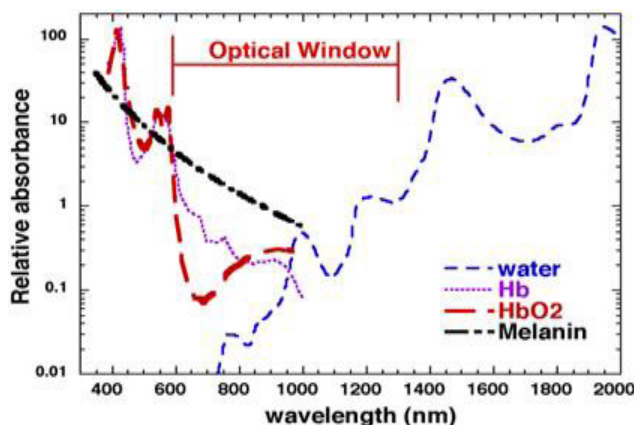
Figure 6. Optimal wavelength in LASER treatment.
Hb= Haemoglobin; HbO2= Oxygenated Haemoglobin
(Source: www.spie.com)

The power of a laser influences treatment time more than it influences the effect of the therapy. The power of a laser is expressed in watts (W). The energy delivered by a laser is expressed in joules (J) (= watt x seconds) per cm². Therefore, the higher the power of a laser (W), the less time is needed to deliver the same amount of energy (J). For example, a laser with double power will need half the time for the same effect (Figure 7).
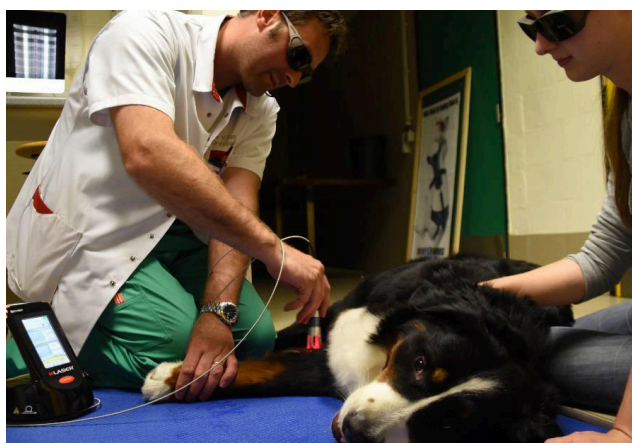
Figure 7. Laser therapy in a dog with chronic elbow arthritis.
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The most frequently advocated indications for laser therapy are wound healing and pain management, although scientific proof is scarce and is often based on in vitro models (Millis et al., 2005).

Studies that involve life animals are limited to laboratory animal studies and reveal carefully positive results concerning wound healing, especially in the early phase of healing (Braverman et al., 1989; Rezende et al., 2007; Peplow et al., 2010). Laboratory animals have also been

used to examine the effects of LLLT on bone healing. Most of these studies have demonstrated a (mild) positive effect when laser therapy was used either alone or in combination with another modality (Pinheiro et al., 2000; Lirani-Galvao et al., 2006; Gerbi et al., 2008). The therapy has shown to be most effective in the early stage of bone healing. (Pinheiro et al., 2000; Queiroga et al., 2008; Batista et al., 2015) Where US therapy is believed to have its optimal effect on bone resorption, LLLT is believed to be responsible for an optimization of bone formation (Lirani-Galvao et al., 2006). The effect on cartilage is dual. Laser therapy shortly after cartilage damage might induce better healing of the lesion (Guzzardella et al., 2000; Morrone et al., 2000; Guzzardella et al., 2001), but there are also indications that it might protect the quality of the cartilage during periods of disuse and immobilization (Akai et al., 1997; Bayat et al., 2004). Whether these effects are similar in other animals is still to be determined.

Several studies in humans and animals have demonstrated that laser therapy reduces pain sensation (Millis et al., 2005; Millis and Levine, 2014). Although the exact mechanism is still unclear, two theories have been proposed. The first theory postulates a release of endorphins and encephalin (Millis et al., 2005; Millis and Levine, 2014). The second theory is based on two studies in rats, where laser therapy induced an inhibitory effect on the conduction of peripheral nerves by inhibiting peripheral nociceptors (Tsuchiya et al., 1994; Wedlock et al., 1996; Wedlock and Shephard, 1996; Chow et al., 2011; Yan et al., 2011).

Laser therapy has also been studied in nerve revalidation with positive effect as well in rat models and in a dog model. This effect was seen in both the acute post-traumatic stage and the more chronic cases of nerve injury. Laser therapy resulted in better functional activity, less scar tissue, decreased degeneration of motor neurons and increased myelinisation and axonal growth. In the dog study, the spinal cord was transected and replaced with a graft. All dogs that received laser therapy walked after nine weeks, while the other dogs remained paralyzed (Rochkind et al., 1986; Rochkind et al., 1987; Shamir et al., 2001; Shin et al., 2003; Rochkind, 2004).

In a study by Draper et al. (2012), the effect of LLLT on the revalidation of T3-L3 disk herniation was investigated in 36 dogs. All dogs underwent hemi-laminectomy. Eighteen of them had additional laser therapy of the

affected region for about five days after surgery. The time to regain mobility of the dogs that received LLLT was three to five days, which was significantly lower than for the dogs that only received surgery (about 14 days). This led to the conclusion that LLLT is beneficial in the revalidation of disk herniation patients (Draper et al., 2012).

LLLT might have benefits on other tissues and conditions, such as tendons, ligaments and osteoarthritis. Although several studies have been performed in human medicine, scientific veterinary literature on this subject is not available for the moment (Millis and Levine, 2014).

Because LLLT uses both visible and invisible light, protective eyewear is required. Heat generated by laser light may damage the retina. Therefore, caution should be taken while operating the laser and treating tissues in the region of the eyes. For the same reason, laser should not be used over growth plates, malignancies and in pregnant patients (Millis and Levine, 2014; Prydie and Hewitt, 2015).

Other modalities

Over the last years, a static or electromagnetic field has been advocated to be beneficial in revalidation therapy. The main focus of this modality is (OA) pain reduction, although it is claimed to have some positive effects on wound and bone healing as well (Millis and Levine, 2014).

Evidence to support these statements is scarce to absent. The limited proof that may be found in the literature is often based on a single study with a limited number of patients (Khanaovitz et al., 1994; Scardino et al., 1998; Sullivan et al., 2013).

Before considering this therapy as a standalone treatment, more peer-reviewed, well-designed studies are needed.

Therapeutic exercises

Exercise is an important factor in rehabilitation. Passive movement and modalities are a great aid, but natural muscle stimulation is still the best way to exercise muscles. A good physio-therapeutic protocol should therefore be composed out of a combination of manual therapy, modalities and therapeutic exercises (Bockstahler et al., 2004).

It is not the objective of this review to discuss every type of exercise. These are well described in the literature

(Bockstahler et al., 2004; Sharp, 2008; Millis and Levine, 2014; Prydie and Hewitt, 2015).

Therapeutic exercises can be divided into three groups: passive, assisted and active exercises. The best-known therapeutic exercise is hydrotherapy, which will be discussed more extensively.

Passive exercises

These are exercises in which the animal does not actively use its own muscles. Examples are passive range of motion (pROM) and stretching exercises. The goal of these exercises is to facilitate the joints ROM and soft tissues flexibility (Bockstahler et al., 2004).

Several studies have indicated that PROM exercises following shortly after injury or surgery has a beneficial effect on the desired outcome (Olson, 1987; Schollmeier et al., 1994; Schollmeier et al., 1996; Millis et al., 1997; Crook et al., 2007; Jandi and Schulman, 2007).

Assisted exercises

In these exercises, the animals use their own muscle strength, while being supported by an aid or by a physiotherapist (Figure 8). These exercises are useful in weight bearing and proprioceptive training (Bockstahler et al., 2004). An example is balance board exercises (Figure 9).

Figure 8. Assisted walking device used in a dog with surgically treated Wobbler's syndrome.
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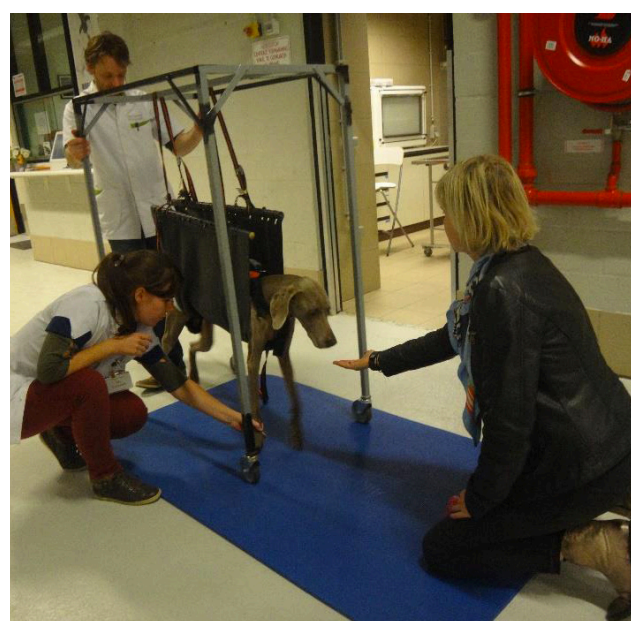*Figure 9. Balance board exercise.*
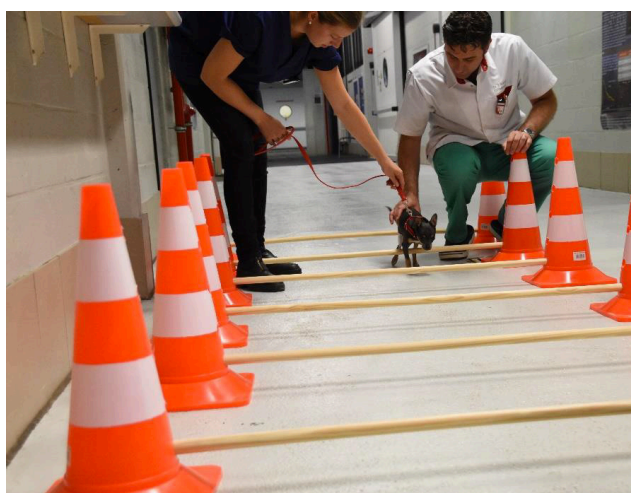*© Dept of Medical Imaging and Small Animal Orthopaedics, Ghent University*

Figure 10. Cavaletti rail.
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Active exercises

Animals perform exercises using only their own muscle strength without any assistance. Known examples are Cavaletti rails and aquatic therapy (Bockstahler et al., 2004) (Figure 10).

Aquatic therapy
One of the most popular exercises in veterinary physiotherapy is aquatic therapy. The reason for its success can be found in the properties of water:

Relative density
Relative density stands for the ratio of the weight of an object, relative to the weight of an equal amount of water (Haralson, 1986). It determines whether an object will either float or sink in the water. Density of an object is expressed in an exact number known as the 'specific gravity'. The specific gravity of water is 1 (Hecox et al.,

1994). This means that if the ratio of the specific gravity of an object to water is more than 1, the object will have the tendency to sink, whereas objects with a ratio of less than 1 will have the tendency to float.
The specific gravity also determines which volume of the object will be submerged. For example, an object with a specific gravity of 0.95 will be submerged for 95%, while 5% will float above the surface (Hecox et al., 1994).

Buoyance
This is the upward thrust of water acting on a body that creates an apparent decrease in the weight of a body while immersed (Hecox et al., 1994). A study in dogs demonstrated that the weight borne immersed in water relative to the weight on the ground was 91% when the water reached the level of the tibial malleoli, 85% when the level reached the femoral condyles, and 38% when the water reached the level of the greater trochanter of the femur (Levine et al., 2010). This results in more comfortable movements with less pain (Bockstahler et al., 2004). Buoyance together with the viscosity also assists in stabilizing less stable dogs, for example in cases of paresis or obesity (Millis and Levine, 2014).

Hydrostatic pressure
At a given depth, the pressure exerted by a liquid on a body is equally divided on all surfaces of that body, i.e. Pascal's law (Polyanin and Chernoutsan, 2010). Consequently, the deeper the body is submerged in the water, the higher the pressure on the body. This might facilitate movements with less pain and reduce oedema and swelling (Millis and Levine, 2014).

Viscosity
Molecules in a liquid have higher cohesive forces than the molecules in a gas. Therefore, the resistance to move in a liquid, such as water, is higher than the resistance to move in air (Geigle et al., 1997). Exercising in water therefore stimulates the muscle function and cardiovascular fitness (Millis and Levine, 2014). Studies in human medicine have demonstrated a positive effect on muscle strength, muscle endurance cardiorespiratory endurance, agility and ROM combined with a reduction in pain (Whitley and Schoene, 1987; Speer et al., 1993; Millis and Levine, 2014). In veterinary medicine, studies are limited. One study has demonstrated an increased ROM after swimming in dogs with a surgically treated cruciate ligament rupture. These dogs also showed an 18.5% higher peak vertical force than dogs that had no post-operative hydrotherapy (Marsolais et al., 2003).

A recent study has demonstrated that hydrotherapy has a beneficial effect on inflammatory biomarkers in dogs. Fifty-five dogs were divided into three groups: healthy dogs without hydrotherapy, dogs with hip OA with hydrotherapy, healthy dogs with hydrotherapy. The dogs were allowed to swim for twenty minutes three times a day for an eight-weeks period. Every two weeks, blood samples were collected to determine specific OA biomarkers. Starting from six weeks, OA dogs showed less pain on clinical examination and there was a significant change in biomarkers. It was concluded that swimming is beneficial for the treatment of dogs with hip OA (Nganvongpanit et al., 2014).

In small animal rehabilitation, aquatic therapy is performed either in a pool or in an underwater treadmill. An underwater treadmill allows a better control of the treatment goals by altering the height of the water and the speed of motion (Figure 11). No life vest is required, although support might be useful when the animals are still slightly unstable in their movement. When necessary, the physiotherapist can enter the treadmill along with the dog.

Figure 11. Underwater treadmill for dogs.
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A pool requires more space and life vests for the patient. The physiotherapist is always required to enter the pool with the dog. A treadmill can be incorporated into the pool.

Hydrotherapy may be used in some stadia of the rehabilitation of nearly all conditions. Because of its influence on musculature and the cardiovascular system, aquatic therapy also helps in training healthy dogs to improve their general condition (Millis and Levine, 2014).

Conclusions

Animal physiotherapy is to be considered in every orthopaedic or neurological condition that causes pain and/or discomfort or dysfunction. Most of the techniques are based on human studies and more recently, some veterinary studies have been published. Because of the versatility of therapy, it is not always easy to attribute clinical progression exclusively to one technique in physiotherapy (or even to physiotherapy itself). Based on the current literature, it can be concluded that there are strong indications that physiotherapy aids in the rehabilitation of clinical patients, whether it is used as pain relief or for intense mobility revalidation.

References

Akai M., Usuba M., Maeshima T., Shirasaki Y., Yasuoka S. (1997). Laser's effect on bone and cartilage change induced by joint immobilization: An experiment with animal model. *Lasers in Surgery and Medicine* 21, 480-484

Anderson A. F., Lipscomb A. B. (1989). Analysis of rehabilitation techniques after anterior cruciate reconstruction. *The American Journal of Sports Medicine* 17, 154-160

Aure O. F., Nilsen J. H., Vasseljen O. (2003). Manual therapy and exercise therapy in patients with chronic low back pain: a randomized, controlled trial with 1-year followup. *Spine* (Phila Pa 1976) 28, 525-532

Batista J. D., Sargenti-Neto S., Dechichi P., Rocha F. S., Pagnoncelli R. M. (2015). Low-level laser therapy on bone repair: is there any effect outside the irradiated field? *Lasers in Medical Science* 30, 1569-1574

Bayat M., Ansari A., Hekmat H. (2004). Effect of low-power helium-neon laser irradiation on 13-week immobilized articular cartilage of rabbits. *Indian Journal of Experimental Biology* 42, 866-870

Bockstahler B., Levine D., Millis D. L., Wandrey S. O. N. 2004. In: B. Egner (editor). Essential Facts of Physiotherapy in Dogs and Cats: Rehabilitation and Pain Management : a Reference Guide with DVD. 1st Edition, BE Vet Verlag, Babenhausen.

Bocobo C., Fast A., Kingery W., Kaplan M. (1991). The effect of ice on intra-articular temperature in the knee of the dog. *American Journal of Physical Medicine and Rehabilitation* 70, 181-185

Braverman B., Mccarthy R. J., Ivankovich A. D., Forde D. E., Overfield M., Bapna M. S. (1989). Effect of heliumneon and infrared-laser irradiation on wound-healing in rabbits. *Lasers in Surgery and Medicine* 9, 50-58

Chow R., Armati P., Laakso E. L., Bjordal J. M., Baxter G. D. (2011). Inhibitory effects of laser irradiation on peripheral mammalian nerves and relevance to analgesic effects: A systematic review. *Photomedicine and Laser Surgery* 29, 365-381

Crook T., McGowan C., Pead M. (2007). Effect of passive stretching on the range of motion of osteoarthritic joints in 10 labrador retrievers. *Veterinary Record* 160, 545-547

Dahlberg J., Fitch G., Evans R. B., McClure S. R., Conzemius M. (2005). The evaluation of extracorporeal shockwave therapy in naturally occurring osteoarthritis of the stifle joint in dogs. *Veterinary and Comparative Orthopaedics and Traumatology* 18, 147-152

de Sousa V. L., de Alvarenga J., Filho J. G. P., Canola J. C., Ferrigno C. R. A., Alves J. M., Duarte L. R. (2008). Low-intensity pulsed ultrasound in diaphyseal fractures: clinical application in dogs. *Ciencia Rural* 38, 1030-1037

Denis M., Moffet H., Caron F., Ouellet D., Paquet J., Nolet L. (2006). Effectiveness of continuous passive motion and conventional physical therapy after total knee arthroplasty: a randomized clinical trial. *Physical Therapy* 86, 174-185

Dias R. C., Dias J. M., Ramos L. R. (2003). Impact of an exercise and walking protocol on quality of life for elderly people with OA of the knee. *Physiotherapy Research International* 8, 121-130

Diong J., Allen N. and Sherrington C. (2016). Structured exercise improves mobility after hip fracture: a metaanalysis with meta-regression. *British Journal of Sports Medicine* 50, 346-355

Draper W. E., Schubert T. A., Clemmons R. M., Miles S. A. (2012). Low-level laser therapy reduces time to ambulation in dogs after hemilaminectomy: a preliminary study. *Journal of Small Animal Practice* 53, 465-469

Drygas K. A., McClure S. R., Goring R. L., Pozzi A., Robertson S. A., Wang C. (2011). Effect of cold compression therapy on postoperative pain, swelling, range of motion, and lameness after tibial plateau leveling osteotomy in dogs. *Journal of the American Veterinary Medical Association* 238, 1284-1291

Dyson M., Pond J. B., Joseph J. and Warwick R. (1968). The stimulation of tissue regeneration by means of ultrasound. *Clinical Science* 35, 273-285

Esnouf A., Wright P. A., Moore J. C., Ahmed S. (2007). Depth of penetration of an 850nm wavelength low level laser in human skin. *Acupuncture & Electro-Therapeutics Research* 32, 81-86

Favaro-Pipi E., Bossini P., de Oliveira P., Ribeiro J. U., Tim C., Parizotto N. A., Alves J. M., Ribeiro D. A., de Araujo H. S. S., Renno A. C. M. (2010). Low-intensity pulsed ultrasound produced an increase of osteogenic genes expression during the process of bone healing in rats. *Ultrasound in Medicine and Biology* 36, 2057-2064

Fox S. M. (2013). Functional physiology of pain. In: D. B. Fox (editor). Pain Management in Small Animal Medicine. First edition, CRC Press, Boca Raton, p. 47 - 90.

Gallagher A., Cross A. R., Sepulveda G. (2012). The effect of shock wave therapy on patellar ligament desmitis after tibial plateau leveling osteotomy. *Veterinary Surgery* 41, 482-485

Geigle P. R., Cheek W. L., Gould M. L., Hunt H. C., Shafic B. (1997). Aquatic physical therapy for balance: the interaction of somatosensory and hydrodynamic principles. *Journal of Aquatic Physical Therapy* 5, 7

Gerbi M. E. M., Marques A. M. C., Ramalho L. M. P., Ponzi E. A. C., Carvalho C. M., Santos R. D. C., Oliveira P. C., Noia M., Pinheiro A. L. B. (2008). Infrared laser light further improves bone healing when associated with bone morphogenic proteins: An in vivo study in a rodent model. *Photomedicine and Laser Surgery* 26, 55-60

Guzzardella G. A., Morrone G., Torricelli P., Rocca M., Tigani D., Brodano G. B., Ferrari D., Fini M., Giardino R. (2000). Assessment of low-power laser biostimulation on chondral lesions: An "in vivo" experimental study. *Artificial Cells, Blood Substitutes and Biotechnology* 28, 441-449

Guzzardella G. A., Tigani D., Torricelli P., Fini M., Martini L., Morrone G., Giardino R. (2001). Low-power diode laser stimulation of surgical osteochondral defects: Results after 24 weeks. *Artificial Cells, Blood Substitutes and Biotechnology* 29, 235-244

Haralson K. (1986). Therapeutic pool programs. Journal of Clinical Outcome Management 5, 7 Hecox B., Mehreteab T. A., Weisberg J. (1994). In: B. Hecox, T. A. Mehreteab, J. Weisberg (editors). Physical Agents: A Comprehensive Text for Physical Therapists. First edition, Appleton & Lange, East Norwalk, Conn.

Hertling D., Kessler R. M. (2006). In: D. J. Payne, A. P. Williams (editors). Management of Common Musculoskeletal Disorders: Physical Therapy Principles and Methods. Fourth edition, Lippincott Williams & Wilkins, Philadelphia.

Jandi A. S., Schulman A. J. (2007). Incidence of motion loss of the stifle joint in dogs with naturally occurring cranial cruciate ligament rupture surgically treated with tibial plateau leveling osteotomy: Longitudinal clinical study of 412 cases. *Veterinary Surgery* 36, 114-121

Johnson J. M., Johnson A. L., Pijanowski G. J., Kneller S. K., Schaeffer D. J., Eurell J. A., Smith C. W., Swan K. S. (1997). Rehabilitation of dogs with surgically treated cranial cruciate ligament-deficient stifles by use of electrical stimulation of muscles. *American Journal of Veterinary Research* 58, 1473-1478

Kathmann I., Cizinauskas S., Doherr M. G., Steffen F., Jaggy A. (2006). Daily controlled physiotherapy increases survival time in dogs with suspected degenerative myelopathy. *Journal of Veterinary Internal Medicine* 20, 927-932

Khanaovitz N., Arnoczky S. P., Nemzek J., Shores A. (1994). The effect of electromagnetic pulsing on posterior lumbar spinal fusions in dogs. *Spine* 19, 705-709

Kieves N. R., MacKay C. S., Adducci K., Rao S., Goh C., Palmer R. H., Duerr F. M. (2015). High energy focused shock wave therapy accelerates bone healing A blinded, prospective, randomized canine clinical trial. *Veterinary and Comparative Orthopaedics and Traumatology* 28, 425-432

Kolarova H., Ditrichova D., Wagner J. (1999). Penetration of the laser light into the skin in vitro. *Lasers in Surgery and Medicine* 24, 231-235

Kristiansen T. K., Ryaby J. P., McCabe J., Frey J. J., Roe L. R. (1997). Accelerated healing of distal radial fractures with the use of specific, low-intensity ultrasound. A multicenter, prospective, randomized, double-blind, placebo-controlled study. *Journal of Bone and Joint Surgery*. American Volume 79, 961-73

Landrum E. L., Kelln C. B., Parente W. R., Ingersoll C. D., Hertel J. (2008). immediate effects of anterior-to-posterior talocrural joint mobilization after prolonged ankle immobilization: a preliminary study. *Journal of Manual and Manipulative Therapy* 16, 100-105

Levine D., Marcellin-Little D. J., Millis D. L., Tragauer V., Vet M. M., Osborne J. A. (2010). Effects of partial immersion in water on vertical ground reaction forces and weight distribution in dogs. *American Journal of Veterinary Research* 71, 1413-1416

Levine D., Millis D. (2002). The effect of TENS on osteoarthritic pain in the stifle of dogs. In: Proceedings of the Second International Symposium on Rehabilitation and Physical Therapy in Veterinary Medicine 14, Knoxville, TN, USA

Levine D., Millis D. L., Marcellin-Little D. J. (2005). Introduction to veterinary physical rehabilitation. *Veterinary Clinics of North America. Small Animal Practice* 35, 1247-1254, vii

Levine D., Millis D. L., Mynatt T. (2001). Effects of 3.3- MHz ultrasound on caudal thigh muscle temperature in dogs. *Veterinary Surgery* 30, 170-174

Lirani-Galvao A. P., Jorgetti V., da Silva O. L. (2006). Comparative study of how low-level laser therapy and low-intensity pulsed ultrasound affect bone repair in rats. *Photomedicine and Laser Surgery* 24, 735-740

Loonam J. E., Millis D., Stevens M. (2003). The effect of therapeutic ultrasound on tendon healing and extensibility. In: Proceedings of the 30th Veterinary Orthopedic Society, Steamboat Springs, Colorado, USA 69

Marsolais G. S., McLean S., Derrick T., Conzemius M. G. (2003). Kinematic analysis of the hind limb during swimming and walking in healthy dogs and dogs with surgically corrected cranial cruciate ligament rupture. *Journal of the American Veterinary Medical Association* 222, 739-743

Maxwell L. (1992). Therapeutic ultrasound: its effects on the cellular and molecular mechanisms of inflammation and repair. *Physiotherapy* 78, 421-426

Melzack R., Wall P. D. (1965). Pain mechanisms - a new theory. *Science* 150, 971-979

Michlovitz S. L. (1996). In: S. L. Michlovitz (editor). Thermal Agents in Rehabilitation. Third edition, F.A. Davis, Philadelphia.

Millard R. P., Towle-Millard H. A., Rankin D. C., Roush J. K. (2013). Effect of cold compress application on tissue temperature in healthy dogs. *American Journal of Veterinary Research* 74, 443-447

Millard R. P., Towle-Millard H. A., Rankin D. C., Roush J. K. (2013). Effect of warm compress application on tissue temperature in healthy dogs. *American Journal of Veterinary Research* 74, 448-451

Millis D., Drum M., Whitlock D. (2011). Complementary use of extracorporeal Shock Wave therapy on elbow osteoarthritis in dogs. *Veterinary Orthopedic Society, Vet Comp Orthop Traumatol* 24(3):A1, 2011

Millis D., Levine A. D., Brumlow M., Weigel J. P. (1997). Scientific presentation abstracts: the seventh annual american college of veterinary surgeons symposium October 1997, Orlando, FL. *Veterinary Surgery* 26, 408-435

Millis D., Levine D. (2014). In: D. Millis, D. Levine (editors). Canine Rehabilitation and Physical Therapy. Second edition, Elsevier Health Sciences, Philadelphia.

Millis D. L. (2004). Getting the dog moving after surgery. *Journal of the American Animal Hospital Association* 40, 429-436

Millis D. L., Francis D., Adamson C. (2005). Emerging modalities in veterinary rehabilitation. *Veterinary Clinics of North America-Small Animal Practice* 35, 1335 -1355

Mlacnik E., Bockstahler B. A., Muller M., Tetrick M. A., Nap R. C., Zentek J. (2006). Effects of caloric restriction and a moderate or intense physiotherapy program for treatment of lameness in overweight dogs with osteoarthritis. *Journal of the American Veterinary Medical Association* 229, 1756-1760

Moffet H., Collet J. P., Shapiro S. H., Paradis G., Marquis F., Roy L. (2004). Effectiveness of intensive rehabilitation on functional ability and quality of life after first total knee arthroplasty: A single-blind randomized controlled trial. *Archives of Physical Medicine and Rehabilitation* 85, 546-556

Morrone G., Guzzardella G. A., Torricelli P., Rocca M., Tigani D., Brodano G. B., Fini M., Giardino R. (2000). Osteochondral lesion repair of the knee in the rabbit after low-power diode Ga-Al-As laser biostimulation: An experimental study. *Artificial Cells, Blood Substitutes and Biotechnology* 28, 321-336

Mosselmans L. (2011). Het effect van ultrageluid op botheling bij de hond. Pilootstudie uitgevoerd bij 5 honden. Department of Medical Imaging and Small Animal Orthopedics, Ghent University, Ghent.

Mosselmans L., Samoy Y., Verleyen P., Herbots P., Van Ryssen B. (2013). Applications of ultrasound in veterinary medicine. *Vlaams Diergeneeskundig Tijdschrift* 82, 103-111

Mueller M., Bockstahler B., Skalicky M., Mlacnik E. and Lorinson D. (2007). Effects of radial shockwave therapy on the limb function of dogs with hip osteoarthritis. V*eterinary Record* 160, 762-765

Nganvongpanit K., Tanvisut S., Yano T., Kongtawelert P. (2014). Effect of swimming on clinical functional parameters and serum biomarkers in healthy and osteoarthritic dogs. *International Scholarly Research Notices: Veterinary Sciences,* 459809

Olson J. E., Stravino V. D. (1972). A review of cryotherapy. *Physical Therapy* 52, 840-853

Olson V. L. (1987). Evaluation of joint mobilization treatment - a method. *Physical Therapy* 67, 351-356

Ostelo R. W., Costa L. O., Maher C. G., de Vet H. C., van Tulder M. W. (2009). Rehabilitation after lumbar disc surgery: an update Cochrane review. *Spine* (Phila Pa 1976) 34, 1839-1848

Ostelo R. W., de Vet H. C., Waddell G., Kerckhoffs M. R., Leffers P., van Tulder M. (2003). Rehabilitation following first-time lumbar disc surgery: a systematic review within the framework of the cochrane collaboration *Spine* (Phila Pa 1976) 28, 209-218

Peplow P. V., Chung T. Y., Baxter G. D. (2010). Laser photobiomodulation of wound healing: a review of experimental studies in mouse and rat animal models. *Photomedicine and Laser Surgery* 28, 291-325

Pinheiro A. L. B., Oliveira M. G., Martins P. P. M., Ramalho L. M. P., Oliveira M. A. M. d., Júnior A. N., Nicolau R. A. (2000). Biomodulatory effects of LLLT on bone regeneration. *Laser Therapy* 13, 73-79

Polyanin A. D., Chernoutsan A. I. (2010). E4 Hydrodynamics. In: A. D. Polyanin and A. I. Chernoutsan (editors). A Concise Handbook of Mathematics, Physics, and Engineering Sciences. 1st ed., CRC Press, Boca Raton, p. 773.

Prydie D., Hewitt I. (2015). In: D. Prydie, I. Hewitt (editors). Practical Physiotherapy for Small Animal Practice. First edition, Wiley, Chichester.

Queiroga A. S., Sousa F. B., Araujo J. M. S., Santos S. D., Sousa C. D. S., Quintans T. C., Almeida T. P., Nonaka C. F. W., Batista L. V. and Limeira F. A. (2008). Evaluation of bone repair in the femur of rats submitted to laser therapy in different wavelengths: An image segmentation method of analysis. *Laser Physics* 18, 1087-1091

Rantanen J., Thorsson O., Wollmer P., Hurme T., Kalimo H. (1999). Effects of therapeutic ultrasound on the regeneration of skeletal myofibers after experimental muscle. *American Journal of Sports Medicine* 27, 54-59

Rawool N. M., Goldberg B. B., Forsberg F., Winder A. A., Hume E. (2003). Power Doppler assessment of vascular changes during fracture treatment with low-intensity ultrasound. *Journal of Ultrasound in Medicine* 22, 145-153

Rexing J., Dunning D., Siegel A. M., Knap K., Werbe B. (2010). Effects of cold compression, bandaging, and microcurrent electrical therapy after cranial cruciate ligament repair in dogs. *Veterinary Surgery* 39, 54-58

Rezende S. B., Ribeiro M. S., Nunez S. C., Garcia V. G., Maldonado E. P. (2007). Effects of a single near-infrared laser treatment on cutaneous wound healing: Biometrical and histological study in rats. *Journal of Photochemistry and Photobiology B-Biology* 87, 145-153

Rochkind S. (2004). The role of laser phototherapy in nerve tissue regeneration and repair: research development with perspective for clinical application. In: Proceedings of the World Association of Laser Therapy, Saõ Paulo, Brazil, 94-95

Rochkind S., Barrnea L., Razon N., Bartal A., Schwartz M. (1987). Stimulatory effect of he-ne low-dose laser on injured sciatic-nerves of rats. N*eurosurgery 2*0, 843-847

Rochkind S., Nissan M., Razon N., Schwartz M., Bartal A. (1986). Electrophysiological effect of hene laser on normal and injured sciatic-nerve in the rat. *Acta Neurochirurgica* 83, 125-130

Rodrigues M. E. S., Kodama A. C., Rollo J. M. D. A., Nascimento A. P. C., Malosso T. G. (2004). Low-intensity pulsed ultrasound effects on a bone model of ovariectomized female rats. *Osteoporosis International* 15, 117

Saunders D. G., Walker J. R., Levine D. (2005). Joint mobilization. Veterinary Clinics of North America-Small *Animal Practice* 35, 1287-1316

Scardino M. S., Swaim S. F., Sartin E. A., Steiss J. E., Spano J. S., Hoffman C. E., Coolman S. L., Peppin B. L. (1998). Evaluation of treatment with a pulsed electromagnetic field on wound healing, clinicopathologic variables, and central nervous system activity of dogs. *American Journal of Veterinary Research* 59, 1177-1181

Schollmeier G., Sarkar K., Fukuhara K., Uhthoff H. K. (1996). Structural and functional changes in the canine shoulder after cessation of immobilization. *Clinical Orthopaedics and Related Research* 310-315

Schollmeier G., Uhthoff H. K., Sarkar K., Fukuhara K. (1994). Effects of immobilization on the capsule of the canine glenohumeral joint - a structural-functional study. *Clinical Orthopaedics and Related Research* 37-42

Schortinghuis J., Ruben J. L., Raghoebar G. M., Stegenga B. (2004). Ultrasound to stimulate mandibular bone defect healing: A placebo-controlled single-blind study in rats. *Journal of Oral and Maxillofacial Surgery* 62, 194- 201

Shamir M. H., Rochkind S., Sandbank J., Alon M. (2001). Double-blind randomized study evaluating regeneration of the rat transected sciatic nerve after suturing and postoperative low-power laser treatment. *Journal of Reconstructive Microsurgery* 17, 133-137

Sharp B. (2008). Physiotherapy in small animal practice. *In Practice* 30, 190-199

Shelbourne K. D., Nitz P. (1990). Accelerated rehabilitation after anterior cruciate ligament reconstruction. *American Journal of Sports Medicine* 18, 292-299

Shelbourne K. D., Wilckens J. H., Mollabashy A., DeCarlo M. (1991). Arthrofibrosis in acute anterior cruciate ligament reconstruction: The effect of timing of reconstruction and rehabilitation. *The American Journal of Sports Medicine* 19, 332-336

Sherrington C., Lord S. R. (1997). Home exercise to improve strength and walking velocity after hip fracture: a randomized controlled trial. *Archives of Physical Medicine and Rehabilitation* 78, 208-212

Shin D. H., Lee E., Hyun J. K., Lee S. J., Chang Y. P., Kim J. W., Choi Y. S., Kwon B. S. (2003). Growth-associated protein-43 is elevated in the injured rat sciatic nerve after low power laser irradiation. *Neuroscience Letters* 344, 71-74

Speer K. P., Cavanaugh J. T., Warren R. F., Day L. and Wickiewicz T. L. (1993). A Role for Hydrotherapy in Shoulder Rehabilitation. *American Journal of Sports Medicine* 21,850-853

Steiss J. E., Levine D. (2005). Physical agent modalities. *Veterinary Clinics of North America-Small Animal Practice* 35, 1317-1333

Sullivan M. O., Gordon-Evans W. J., Knap K. E., Evans R. B. (2013). Randomized, controlled clinical trial evaluating the efficacy of pulsed signal therapy in dogs with osteoarthritis. *Veterinary Surgery* 42, 250-254

Tanzer M., Kantor S., Bobyn J. D. (2001). Enhancement of bone growth into porous intramedullary implants using non-invasive low intensity ultrasound. *Journal of Orthopaedic Research* 19, 195-199

Toy E., Ozturk F., Altindis S., Kozacioglu S., Toy H. (2014). Effects of low-intensity pulsed ultrasound on bone formation after the expansion of the inter-premaxillary suture in rats: a histologic and immunohistochemical study. *Australian Orthodontic Journal* 30, 176-183

Tsuchiya K., Kawatani M., Takeshige C., Matsumoto I. (1994). Laser irradiation abates neuronal responses to nociceptive stimulation of rat-paw skin. *Brain Research Bulletin* 34, 369-374

Wakim K. G., Porter A. N., Krusen F. H. (1951). Influence of physical agents and of certain drugs on intra-articular temperature. *Archives of Physical Medicine and Rehabilitation* 32, 714-721

Wedlock P., Shephard R. A., Little C., McBurney F. (1996). Analgesic effects of cranial laser treatment in two rat nociception models. *Physiology & Behavior* 59, 445-448

Wedlock P. M., Shephard R. A. (1996). Cranial irradiation with GaAlAs laser leads to naloxone reversible analgesia in rats. *Psychological Reports* 78, 727-731

Whitley J. D., Schoene L. L. (1987). Comparison of heartrate responses - water walking versus treadmill walking. *Physical Therapy* 67, 1501-1504

Yan W. X., Chow R., Armati P. J. (2011). Inhibitory effects of visible 650-nm and infrared 808-nm laser irradiation on somatosensory and compound muscle action potentials in rat sciatic nerve: implications for laser-induced analgesia. *Journal of the Peripheral Nervous System* 16, 130-135

Zeilhofer H. U., Wildner H., Yevenes G. E. (2012). Fast synaptic inhibition in spinal sensory processing and pain control. *Physiological Reviews* 92, 193-235

Zink M. C., Van Dyke J. B. 2013. In: M. C. Zink, J. B. Van Dyke (editors). Canine Sports Medicine and Rehabilitation. First edition, Wiley, Iowa.

Zorlu U., Tercan M., Ozyazgan I., Taskan I., Kardas Y., Balkar F., Ozturk F. (1998). Comparative study of the effect of ultrasound and electrostimulation on bone healing in rats. *American Journal of Physical Medicine & Rehabilitation* 77, 427-432

Zusman M. (2010). There's something about passive movement. *Medical Hypotheses* 75, 106-110

Reprint paper*

Fracture healing:
Applications of ultrasound in veterinary medicine

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ABSTRACT

The therapeutic use of ultrasound in human medicine is known to have a positive effect on the healing of tissue and bone. Therefore, ultrasound has frequently been applied in patients with a bone fracture (e.g. Exogen®). In veterinary medicine, several studies have been performed to investigate the effect of ultrasound on bone healing. Although the healing time in several patients was shortened after the application of ultrasound, the technique is not currently routinely applied to cases of delayed or non-union. The literature and existing trials with ultrasound in veterinary medicine are reviewed in this article. The mechanism of action of ultrasound during the various phases of tissue and bone healing are described. Since the interest in physical therapy is increasing, the technique will probably be applied more frequently in companion animals in the near future.

Introduction

Ultrasound waves are sound waves whose frequency is too high to be heard by the human ear. Ultrasound ranges from approximately 18kHz to 800MHz. Ultrasonic vibrations are usually generated by the conversion of electrical or magnetic energy into mechanical energy (Millis and Levine, 1997).

In 1927 it was discovered that ultrasound has a lasting effect on biological systems (Wood and Loomis, 1927). From then on, various studies on the safety of ultrasound and ultrasound use as therapy for multiple musculoskeletal disorders, such as tendonitis, bursitis, muscle contractures and fractures (Wood and Loomis, 1927; Ter Haar, 2007) were conducted.

The effects of ultrasound are traditionally divided into two groups: the thermal and non-thermal effects.

The non-thermal effects accelerate the inflammatory phase, so that the proliferative phase of the repair period will be reached more quickly (Levine et al., 2001). Watson (2006) demonstrated that the non-thermal effects accelerate tissue repair by optimizing the inflammation, proliferation and remodelling phase.

Ultrasound is also responsible for a rise in tissue temperature (Levine et al,. 2001; Fyfe et al., 1985). The magnitude of this increase is dependent on a number of parameters, such as: the intensity of the ultrasound waves, the frequency of the ultrasonic waves and the homeostatic process that seeks to prevent the temperature rise in the tissues (Baker et al, 2001; Steiss, 2003; Watson, 2008). Although this heating effect may be very small (<1° C), some enzymes, such as matrix metalloproteinase-1 (MMP-1) or the collagenase enzyme, are positively enhanced by these minimal temperature rises (Welgus et al., 1981). Matrix metalloproteinase-1 degrades extracellular matrix proteins.

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The stimulation of MMP-1 has a beneficial effect on bone healing since the matrix during the healing process must be partially destroyed.

The application of ultrasound therapy in small animals originates from human medicine. In the past, ultrasound has been used primarily for its thermal effects but in recent years it is becoming increasingly popular for its non-thermal effects, in particular because of the positive effect on tissue and bone healing. In 1994, the United States Food and Drug Administration approved the use of low-intensity ultrasound to accelerate the healing of recent fractures and from 2000, ultrasound was also be used for the treatment of non-unions (Rubin et al., 2001).

However, so far this therapy is not routinely used in veterinary medicine, and is mainly applied in orthopaedic patients with unsatisfactory results following conventional treatment.

A modified form of ultrasound therapy is the extracorporeal shockwave therapy (ESWT). The term 'shockwave' refers to mechanical shock wave pulses, which extend as a wave passes through a medium (water, air or a solid). It involves audible energy sound waves. There are two forms of shockwave used in practice (both in human and in veterinary medicine): radial shockwave (RSWT) and focused shockwave (FSWT). Both techniques work equally well but have a different scope. In devices with radial shockwave, the energy is distributed over a large area. These devices produce a low to medium energy level. In contrast, in devices with focused shockwave, the energy is concentrated in a certain point. These devices produce a high energy level. RSWT can stimulate bone healing (Wang et al., 2001), but will not be discussed in this article.

The purpose of this article is to give an overview of the existing literature on the use of ultrasound in veterinary medicine.

Action mechanism of ultrasound

The ultrasound waves are sent into the body with the aid of a transducer (Figure 1), which must be in direct contact with the skin surface. To minimise reflection of the waves from the transition between the transducer and skin, the animal must be clipped at the site of treatment and a medium must be placed between skin and the transducer (Millis and Levine, 1997). Ideally, this medium is sufficiently

Figure 1. A transducer.
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fluid so that it can cover the entire surface, and is relatively viscous so that it stays in place. In addition, it must allow the transmission of ultrasound waves with the least possible absorption, cushioning or disruption. Media based on water or gel are preferable to media containing oil or cream (Watson, 2008).

There are three possibilities for the application of ultrasound: direct coupling, under-water immersion and gel pads (Millis and Levine, 1997; Steiss, 2003).

1. Direct link

With direct coupling, a water-soluble gel is applied on the skin and to the head of the device. Products, such as electron-conductive gels, lanolin derivatives and mineral oils, are discouraged.

2. Water immersion

In the water immersion method, the treated part of the body is placed in a container with water at room temperature, both the skin and the water must be clean. The distance between the transducer and the skin varies between 0.5 and 3.0 cm. Metal has the property to reflect a portion of the ultrasound waves, so that the intensity is higher in certain places. As a result, preference is given to rubber or plastic containers for the underwater immersion method.

3. Coupling device

In this method a contact pad, such as a water balloon is used. The presence of gel on the contact surface is important in order to minimise reflection (Steiss, 2003).

When the ultrasound waves penetrate the tissue, the energy decrease is directly proportional to the distance travelled as the ultrasound waves are absorbed (Rubin et al., 2001).

The extent of absorption depends on the type of tissue (Figure 2). When ultrasound is used on tissue with a low absorption, the therapy is less effective than when it is applied to tissue with a high absorption (Watson, 2006; Watson, 2008). The absorption is higher in tissues with high-protein levels and few fat cells. This explains why ultrasound waves easily penetrate through subcutaneous fat with little loss of energy and why they are more absorbed by tissues with high levels of collagen (Steiss, 2003; Watson, 2006). The big problem with cartilage and bone is reflection of the ultrasound waves from the surface. This means that a significant portion of the ultrasound energy is reflected and cannot be absorbed.
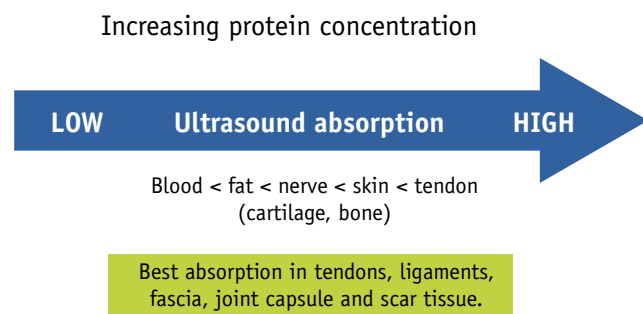
Figure 2. Schematic representation of level of absorption in the various tissues

Ultrasound is generated by a high frequency generator or transducer which contains piezoelectric crystals. These have a special property in that they can be deformed by electrical current and emit sound waves with a frequency inherent to the crystal. In the case of ultrasound, longitudinal sound waves are emitted by the transducer and the deeper tissues are warmed up to 40-45 °C to a maximum depth of 5 cm, without overheating the superficial tissues (Millis and Levine, 1997; Baxter and McDonough, 2007; Watson, 2006) (Fig.3).

If the unit is in continuous mode, both thermal and non-thermal effects occur. In pulsed mode, the non-thermal effects are mainly present.
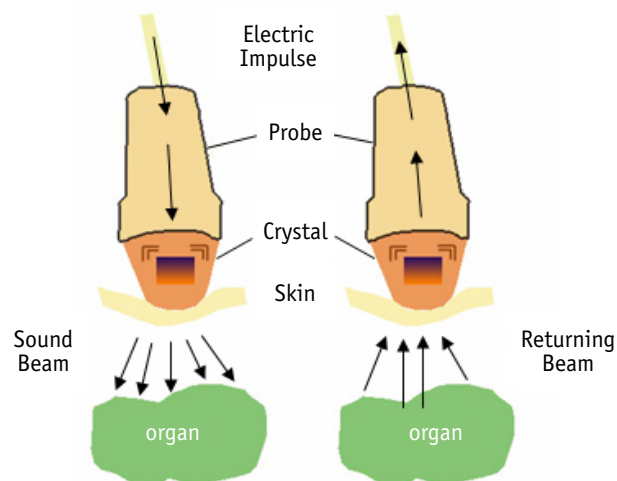
Figure 3. Action mechanism of ultrasound on tissues.

According to Baker et al. (2001), it is incorrect to assume that at a given moment only one effect is present. According to the authors, these two effects are inseparable from each other.

The various stages of ultrasound effect on soft tissue

After damage, soft tissue is repaired through a complex cascade system. This leads to the formation of scar tissue which restores the continuity of the damaged tissue.

The effect of ultrasound during this repair process varies according to the time of application. It is very important to know the stage of the repair process of the tissue at the time of treatment (Watson, 2006).

The bleeding phase

It is not advisable to apply ultrasound immediately after the trauma because this may increase the local blood flow. Since haemorrhage is present, this would be detrimental to the tissues (Watson, 2006) (Figure 4).

The inflammatory phase

During the inflammatory phase ultrasonic waves have a positive influence on mast cells, platelets and white blood cells with a phagocytic role (Nussbaum, 1997; ter Haar, 1999; Fyfe and Chahl, 1982; Maxwell, 1992). Ultrasound waves induce the degranulation of mast cells, which in turn causes the release of arachidonic acid. Arachidonic acid is the precursor of prostaglandins and leukotrienes (Mortimer and Dyson, 1988; Nussbaum, 1997; Leung et al., 2004). By increasing the activity of these cells ultrasound can exert a
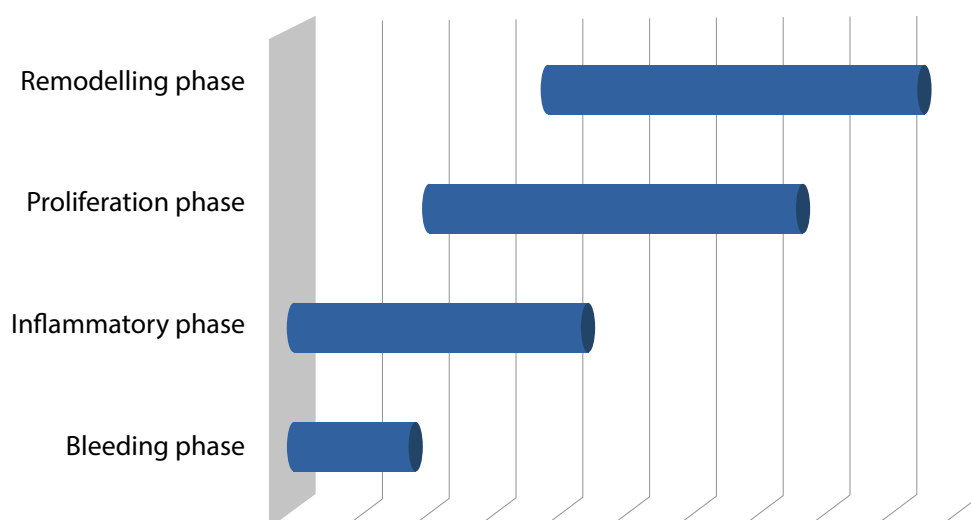
Figure 4. Overview of the stages of bone healing.

pro-inflammatory effect on tissues. The more efficient this process is, the faster the tissue will move on to the next phase, the proliferation phase (Watson, 2006).

The proliferation phase
During this phase, ultrasound waves stimulate fibroblasts, endothelial cells and myofibroblasts (Ramirez et al., 1997; Dyson and Mortimer, 1988; Young and Dyson, 1990; Nussbaum, 1997, and Maxwell, 1992). These cells are present in normal circumstances, during the formation of scar tissue. Just as in the inflammatory phase, ultrasound waves do not induce an increase in the activity of these cells but ensure the highest possible efficiency of the proliferation phase, promoting the formation of scar tissue (Watson, 2006).

Low-intensity pulsed ultrasound (LIPUS) causes an increase in protein synthesis, fibroplasia and improved collagen synthesis (Harvey et al., 1975).

Low-intensity ultrasound only has an effect on soft tissue cells; cells in bone tissue show no response (Romano and Logoluso, 2009).

The remodelling phase
Ultrasound can affect the remodelling of scar tissue. It not only improves the orientation of the newly formed collagen fibres, but allows for a shift of type III to type I collagen, increasing the tensile strength and improving the mobility of scar tissue (Nussbaum, 1998; Wang, 1998).

Ultrasound effect on bone healing

In 1952, Corradi and Cozzolino demonstrated that the callus formation at the site of a radial fracture in rats could be stimulated by the application of continuous wave ultrasound. These findings led to the clinical use of ultrasound in veterinary medicine.

In spite of the advanced techniques available today to treat fractures, around 5 to 10% of all fractures in human medicine do not heal (Brinker and O'Connor, 2008). If the continuity of the bone has not been restored three months after the trauma, it is termed a delayed union or delayed healing. If a fracture has not healed after nine months and no radiological signs of fracture healing are visible during the last three months, it is a non-union (Brinker and O'Connor, 2008).

Risk factors for the development of delayed or non-union include: diabetes, ischaemia and / or infectious processes at the fracture site, severe soft tissue damage and inadequate fixation of the fracture (Romano and Logoluso, 2009).

The different phases of fracture healing
Fracture healing can be divided into direct and indirect fracture healing. The determinants of the type of fracture healing are reduction, apposition and the forces acting on the fracture line tissues (Risselada et al., 2007). In the case of direct fracture healing, no (or only a minimal amount of) external callus will be formed. Mobility at the fracture site is low enough to allow direct bone formation (Brinker 2008; Risselada et al., 2007). An adequate blood supply is needed during this process. If both ends of the bones are in direct contact with each other, then there is contact healing; if there is a gap, then there is gap healing (Brinker, 2008).

Indirect fracture healing occurs in overlapping stages (Figure 5). During the acute phase, a local haematoma is formed at the site of the fracture fragments. At the level of the haematoma, inflammatory mediators and growth factors are
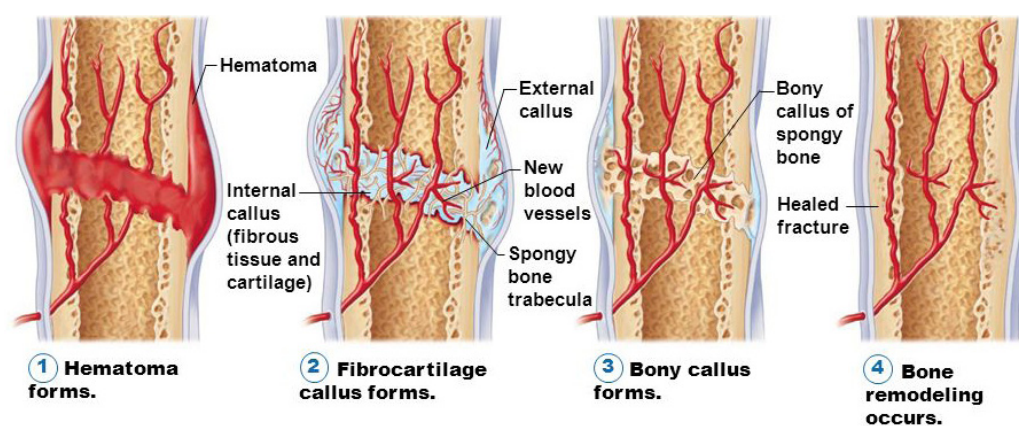
Figure 5. The various stages during indirect fracture healing. (©Pearson education)

released that will stimulate angiogenesis and bone healing (Stage 1) (Claes and Willie, 2007).

Connective tissue cells start to grow in the clot. This is the so-called repair-callus formation (Stage 2). Osteoclasts remove damaged and necrotic tissue and osteoblasts form connective tissue (formation of cartilage and collagen type II), which makes a connection between the two bone fragments (i.e. fibrous callus).

In a further stage, the cartilage undergoes mineralization (i.e. endochondral ossification), and mature collagen (type I) is also formed (stage 3). In this way, a bony callus is created around the site of the bone fracture and the fracture, which initially was completely loose, gradually solidifies. Afterwards, the callus is remodelled for optimal stability (stage 4) (Claes and Willie, 2007).

The type of tissue that is formed in the fracture, depends on the biomechanical characteristics and the forces that are present at the level of the fracture. The most important factor determining the type of tissue, is strain. Strain is defined as the relative change in distance between both fracture ends relative to the original distance between the two (Perren, 2002).

The effect of ultrasound on bone healing

Dyson and Brooks (1983) showed that a treatment with pulsed ultra-sound waves of 500mW/cm², accelerated fracture healing compared to a control group that did not undergo any treatment. It was found that ultrasound therapy had the greatest effect during the initial phases of bone healing.

In the past, there have been several studies conducted to investigate the biological mechanism responsible for the effects of ultrasound on fracture healing. These studies were conducted both in vitro and in vivo.

Low-intensity ultrasound waves cause an increase in calcium incorporation into differentiated cartilage and in cell cultures of bone tissue, which results in a change in cell metabolism. The increased calcium uptake increases the secondary messenger activity. This in turn stimulates adenylate cyclase activity, which promotes the synthesis of TGF-β (transforming growth factor-β) by osteoblasts (Ryaby et al., 1989).

Ultrasound also has a positive effect on the secondary messenger activity in primary chondrocytes. After ultrasound application the release of cellular calcium increases with a frequency of 50mW/cm² (Parvizi et al., 1997). Kokubu et al. (1999) demonstrated that ultrasonic waves with an intensity of 30mW/cm² increase the production of prostaglandin E2 by the induction of the cyclooxygenase-2 m RNA in osteoblasts of mice. Prostaglandin E2 activates the osteoblasts and also the precursor cells from the bone marrow that differentiate into osteoblasts (Kokubu et al., 1999).

Ito et al. (2000) investigated the effect of ultrasound on the secretion of growth factors in a co-culture of human osteoblasts and endothelial cells, and found that ultrasound increases the release of PDGF (platelet-derived growth factor).

When chondrocytes are exposed to low intensity ultrasound waves, the gene expression of aggrecan (chondroitin sulphate proteoglycan 1) is stimulated. This gene expression normally occurs during the early phase of fracture healing. During chondrogenesis, this forms chondroitin aggregates with hyaluronic acid, decorin and biglycan. These aggregates form the basis for type II collagen, which is very important during callus formation (Wu et al., 1996).

Reher et al. (2002) demonstrated the relationship between ultrasound and the increase of nitric oxide (NO) and prostaglandin E2 by exposing human osteoblasts to

ultrasound. For this experiment, they used two types of ultrasound (pulsed 1MHz and non-pulsed 45kHz), and a control group. The osteoblasts, originating from the mandible, were exposed for 5 minutes to both types of ultrasound and were then incubated at 37° C for 24 hours. The NO concentration was measured colorimetrically while the PGE2 was determined by radioimmunoassay. It was found that ultrasound causes a significant increase in both the NO and PGE2 concentrations. Both NO and prostaglandins are necessary for the induction of bone formation and remodelling, caused by mechanical stimuli. Nitric oxide plays an important role in the remodelling as it can regulate both osteoclast and osteoblast activity (Reher et al., 2002). The architecture of trabecular bone is influenced by interfering forces exerted on the bone (= Wolff's law). The shape and architecture of bone tissue adapts to the mechanical forces exerted on it. This is done by remodelling of the bone tissue depending on the magnitude and direction of the forces exerted (Huiskes et al., 2000). Ultrasound waves form a mechanical force which has an effect according to Wolff's law on the remodelling of bone tissue in a non-invasive manner (Rubin et al., 2001).

Recently, a systematic review and meta-analysis were published on the effect of low-intensity pulsed ultrasound (LIPUS) on bone healing. This was based on 23 publications selected by two evaluators on the basis of several criteria (including: application of LIPUS, fractures and human clinical trials). Of the experimental trials on recent fractures, seven were eligible for the meta-analysis. The criterion used was the time at which a radiographic increase was observed with regard to the density and size of the initial periosteal reaction. It was established that bone healing in patients with a recent fracture showed a faster radiographic healing time after treatment with LIPUS.

Although there is weak evidence for low intensity pulsed ultrasonic waves also contributing to radiographic healing of delayed unions and non-unions, it is not possible to analyse the data, due to a lack of sufficient studies with comparable results (Tajali et al., 2012).

Studies performed on dogs

Using power-Doppler ultrasonography, Rawool et al. (2003) were able to visualise changes in vasculature at the fracture site after treatment with LIPUS. In six dogs (divided into two groups), with osteotomy of the ulna performed. From the first day post-operatively, the first group was treated with pulsed ultrasound waves (pulse cycle determination of 20%; 1.5 MHz; intensity: 30 mW/cm²) for twenty minutes per day. Group 2 was a control group. After one week of treatment, vascularity at the fracture site was examined with the aid of power-Doppler ultrasonography. It was found that the vascularization at the fracture site was three times higher in the treated group than in the control group. After eleven days, the difference was still 33%. The use of ultrasound had a positive effect on vascularization at the site of the fracture.

The first day postoperatively, the fracture was clearly visible in both groups, on both the X-ray and the ultrasound images. On the last day, both imaging techniques were repeated, and the fracture in the treated group had started to fuse, while the fracture was still clearly visible in the control group. This suggested that pulsed ultrasound waves have a positive effect on bone healing.

A similar experiment included dogs (of different breeds and with ages ranging from seven months to six years) with recent diaphyseal fractures of the radius, ulna, femur or tibia. Osteosynthesis (intramedullary pinning, external fixation, or a combination of both) was carried out to obtain good stability at the fracture site. Next, the dogs were divided into two groups, one group was treated with ultrasound (LIPUS, 30mW/cm², 1.5MHz, 20% pulse ratio, for 20 minutes per session) and the other group was used as a control group for 21 days. On day 30 postoperatively, bone healing was compared between the two groups. The average bone healing time was found to be significantly lower in the treated group (mean 67.5 days) than in the control group (mean 106 days). This clinical study also confirmed that LIPUS stimulates bone healing in the case of recent diaphyseal fractures (Sousa et al., 2008).

To study the effect of ultrasound on medium to large bone fractures, eight dogs were included in a study and were divided into two groups. In both groups, double ulnar osteotomy was performed under general anaesthesia. The size of the osteotomy was determined by the width (diameter) of the diaphysis. In group 1, this amounted to the half of the diaphysal width, in group 2, the created defect was bigger: one-and-a-half times the width of the diaphysis. The double bilateral osteotomy was performed in all dogs. One randomly chosen leg was treated with ultrasound (LIPUS, 1MHz, 50mW / cm², 20% pulse ratio, 15 minutes) from the first day after surgery for six days. The same procedure was repeated on the other leg, but in this case it ultrasound device was not connected to AC power.

Table 1. Overview of the investigations performed into the effect of ultrasound on bone healing.

Study	Fracture type/Cell type	Administered intensity	Result
In-vitro studies			
Chapman et al., 1980	Thymocytes	0.5-3 W/cm² 2 W/cm²	↓ Intracelullar K+ ions ↓ Intake of K+ ions ↑ K+ efflux
Ryaby et al., 1989	Differentiated cartilage and bone cell culture	200 mW/cm²	↑ Ca+ incorporation
Ryaby et al., 1991, 1992	MC3T3/TE85 osteoblast cell lines	20, 30, 45 mW/cm²	↑ Adenylate cyclase / ↑ TGFβ
Wu et al., 1996	Chondrocytes	50, 120 mW/cm²	↑ Expression aggrecan mRNA
Parvizi et al., 1997, 1999	Chondrocytes	50-500 mW/cm²	↑ Release intracelullar Ca+ ↑ Expression aggrecan mRNA, ↑ Proteoglycane synthesis
Kokubu et al., 1999	MC3T3 osteoblastic cell lines	30 mW/cm²	↑ Expression of PGE2/COX-2
Ito et al., 2000	SaOS-2 cell lines, HUVEC endothelial cells	30 mW/cm²	↑ PDGF-AB secretion
Sun et al., 2001	Osteoblast-osteoclast cell culture	0.068 W/cm²	↑ Number of osteoblasts ↓ Number of osteoclasts ↑ PGE2 and TNFα
In-vivo studies			
Corradi and Cozzolinà, 1952	Radial fracture in rats	Continuous ultrasonic waves	Suspicion of an increased callus formation
Dyson and Brooks, 1983	Radial fracture in rats	Pulsed ultrasonic waves, 500 mW/cm²	Accelerated fracture healing
Tanzer et al., 1996	Porous coated implant in a femoral fracture	30 mW/cm²	Accelerated ingrowth of bone
Yang and Park, 2001	Small and large diaphyseal defects of the ulna	1 MHz, 50 mW/cm² 20% pulse ratio, 15 minutes	↓ Incidence of non-union; accelerated bone healing
Rawool et al., 2003	Osteotomy of the ulna	30 mW/cm²	Increased vascularisation at the site of osteotomy
Sousa et al., 2008	Diaphyseal fracture (radius/ulna/femur/tibia) in the dog	30 mW/cm²	Accelerated bone healing in recent fractures

The bone healing was followed up one, three and five months postoperatively. In the first group there was a significant difference between the treated and untreated front leg. In four dogs remodelling of the cortex had occurred after five months on the treated side, while it was visible only in one patient in the control group at that time. No patient was diagnosed with a non-union.

In group 2, there was also a significant difference. In three patients, there was a non-union in the untreated front leg; this in contrast to the treated paw which showed bone healing in all patients, while the remodelling phase occurred later. The conclusion was that, in the case of small and large diaphyseal defects of the ulna, LIPUS stimulates bone healing and also reduces the incidence of the occurrence of a non-union in large defects of the ulna (Yang and Park , 2001).

Since several studies have found that ultrasound has a positive influence on fracture healing, an experiment was drawn up examining the effect of ultrasound on bone ingrowth into porous coated implants. Twelve dogs were included in the study in which bilateral trans-cortical implants were inserted in the femur. For each dog one femur was treated with ultrasound, the other served as a control. Results showed that the implants treated with ultrasound had an accelerated bone growth compared to the control side

(average increase of 18%) (Tanzer et al., 1996).
As part of a master's thesis (Mosselmans et al., 2011), a pilot study was conducted in five dogs (different breeds, age, type of fracture and type of fixation). In three patients, the presence of a delayed union was the purpose of treatment. In one patient with bilateral elbow incongruence, bilateral ulnotomy, and this patient was treated unilaterally (the non-treated side was used as a control). In the last patient, a recent radioulnar fracture in a plaster cast was treated.

All patients were treated with low intensity pulsed ultrasound waves (intensity = 0.3 W/cm², frequency = 3 MHz and a pulse ratio of 2/8) for 20 minutes per day. The total number of sessions was eight. In order to evaluate the results of this study, use was made of radiographic images, ultrasound images obtained with a linear transducer and a lameness score before and after treatment. In patients with delayed union, the callus was more prominent than in the initial situation. Vascularization had increased in most patients, and the lameness score after treatment had decreased in all but one patient compared to the lameness score prior to treatment. However, due to the limited number of cases and lack of control X-ray images, this pilot study provided no scientific evidence of the positive effect of ultrasound on bone healing in the dog – although there was a clear suspicion (Table 1).

Conclusion

In human medicine, the use of ultrasound in order to stimulate bone healing has already been acknowledged for a long time. In humans, ultrasound devices are often used to stimulate bone healing in the case of a delayed or non-union.

In veterinary medicine, the use of ultrasound is recognised as a positive stimulus for bone healing and several experiments (mainly in rats) have been performed to verify its effect. It can be concluded from most of these trials that ultrasound causes an acceleration of bone healing. The limited studies in dogs show that it induces accelerated vascularization and callus formation.

Despite these positive experiences, ultrasound is not routinely used in veterinary medicine in the event of a delayed or non-union. Possible explanations are that the technology is still too little known, the devices are not sufficiently available and the application is fairly labour intensive. With the increasing popularity of animal physical therapy, the awareness of owners and increasing availability of ultrasound devices, this technology is likely to be more widely accepted in veterinary medicine in future.

References

Baker K.G., Robertson V.J., Duck F.A. (2001). A review of therapeutic ultrasound: Biophysical effects. *Physical Therapy* 81, 1351-1358.

Baxter G.D., McDonough S.M. (2007). Principles of electrotherapy in veterinary physiotherapy. In: McGowan C., Goff L., Stubbs N. (editors). Animal Physiotherapy: Assessment, Treatment and Rehabilitation of Animals. Blackwell Publishing Ltd, Oxford, p. 184-186.

Brinker M.R., O'Conner D.P. (2008). Nonunions: evaluation and treatment. In: Browner B.D., Jupiter J.B., Levine A.M., rafton P.G. en Krettek C. (editors). Skeletal Trauma. 4de Editie, W.B. Saunders Company, p. 1-94.

Chapman I.V., MacNally N.A., Tucker S. (1980). Ultrasound-induced changes in rates of infl ux and effl ux of potassium ions in rat thymocytes in vitro. *Ultrasound in Medicine and Biology* 6, 47-58.

Claes L., Willie B. (2007). The enhancement of bone regeneration by ultrasound. *Progress in Biophysics and Molecular Biology* 93, 384-398.

Cook S.D., Ryaby J.P., McCabe J., Frey J.J., Heckman J.D., Kristiansen T.K. (1997). Acceleration of tibia and distal radius fracture healing in patients who smoke. *Clinical Orthopaedics and related research* 337, 198-207.

Corradi C., Cozzolino A.(1952). The action of ultrasound on the evolution of an experimental fracture in rabbits. *Minerva Ortopedica* 55, 44-45.

Dyson M., Brookes M. (1982). Stimulation of bone repair by ultrasound. *Ultrasound in Medicine & Biology,* vol. 2, 61-66.

Fyfe M.C., Bullock M.I. (1985). Therapeutic ultrasound: some historical background and development in knowledge of its effect on healing. *Australian Journal of Physiotherapy* 31, 220-224.

Fyfe M.C., Chahl L.A. (1982). Mast cell degranulation: A possible mechanism of action of therapeutic ultrasound. *Ultrasound in Medicine and Biology* 8 (Suppl 1), 62.

Harvey W., Dyson M., Pond J.(1975). Metabolic changes induced by ultrasound in fi broblasts in vitro. In: Kazner E., de Vlieger M., Muller H.R., McCready V.R. (editors). Proceedings of the Second European Congress on Ultrasonics in Medicine, Amsterdam, the Netherlands: Excerpta Medica, p. 10-21.

Huiskes R., Ruimerman R., Van Lenthe G.H., Janssen J.D. (2000). Effects of mechanical forces on maintenance and adaptation of form in trabecular bone. *Nature* 405, 704- 706.

Ito M., Azuma Y., Ohta T., Komoriya K. (2000). Effects of ultrasound and 1,25-dihydroxyvitamin D3 on growth factor secretion in co-cultures of osteoblasts and endothelial cells. *Ultrasound in Medicine and Biology* 26, 161-166.

Kokubu T., Matsui N., Fujioka H., Tsunoda M., Mizuno K. (1999). Low intensity pulsed ultrasound exposure increases prostaglandin E2 production via the inductionof cyclooxygenase-2 mRNA in mouse osteoblasts. B*iochemical and Biophysical Research Communications* 256, 284-287.

Leung M.C., Ng G.Y., Yip K.K. (2004). Effect of ultrasound on acute infl ammation of transected medial collateral ligaments. A*rchives of Physical Medicine and Rehabilitation* 85, 963-966.

Levine D., Millis D.L., Mynatt T. (2001). Effects of 3-3- MHz ultrasound on caudal thigh muscle temperature in dogs. *Veterinary Research* 30, 170-174.

Maxwell L. (1992). Therapeutic ultrasound: its effects on the cellular and molecular mechanisms of infl ammation and repair. *Physiotherapy* 78, 421-426.

Millis D.L., Levine D. (1997). The role of exercise and physical modalities in the treatment of osteoarthritis. *Veterinary Clinics of North America: Small Animal Practice* 27, 913-931.

Mortimer A.J., Dyson M. (1988). The effect of therapeutic ultrasound on calcium uptake in fi broblasts. *Ultrasound in Medicine and Biology* 14(6), 499-506.

Mosselmans L., Samoy Y., Verleyen P., Herbots P., Van Ryssen B. (2011). Het effect van ultrageluid op botheling bij de hond. Pilootstudie uitgevoerd bij 5 honden. Master's thesis, Faculteit Diergeneeskunde, UGent.

Nussbaum E.L. (1997). Ultrasound: to heat or not to heat – that is the question. *Physical Therapy Reviews* 2, 59-72.

Parvizi J., Parpura J., Greenleaf J.F., Bolander M.E. (2002). Calcium signalling is required for ultrasound stimulated aggrecan synthesis by rat chondrocytes. *Journal of Orthopaedic Research* 20, 51-57.

Perren S.M. (2002). Evolution of the internal fi xation of long bone fractures. The scientifi c basis of biological internal fi xation: choosing a new balance between stability and biology. *The Journal of Bone and Joint Surgery* 84, 1093-1110.

Ramirez A., Schwane J.A., McFarland C., Starcher B (1997). The effect of ultrasound on collagen synthesis and fi broblast proliferation in vitro. *Medicine and Science in Sports and Exercise* 29, 326-332.

Rawool D., Goldberg B., Forsberg F., Winder A., Talish R., Hume E. (2003). Power Doppler assessment of vascular changes during fracture treatment with low-intensity ultrasound. *Journal of Ultrasound in Medicine* 22(2), 145-153.

Reher P., Harris M., Whiteman M., Hai H.K. en Meghji S. (2002). Ultrasound stimulates nitric oxide and prostaglandin E2 production by human osteoblasts. *Bone* 31, 236-241.

Risselada M., van Bree H., Kramer M., Duchateau L., Verleyen P., Saunders J.H. (2007). Ultrasonographic assessment of fracture healing after plate osteosynthesis. *Veterinary Radiology & Ultrasound* 4*8*, 368-372.

Romano C.L., Logoluso N. (2009). Low-intensity pulsed ultrasound for the treatment of bone delayed union or nonunion : a review. *Ultrasound in Medicine and Biology* 35, 529-536.

Rubin C., Bolander M., Ryaby J.P., Hadjiargyrou M. (2001). The use of low-intensity ultrasound to accelerate the healing of fractures. *The Journal of Bone and Joint Surgery* 83A, 259-270.

Ryaby J.T., Bachner E.J., Bendo J.A., Dalton P.F., Tannenbaum S., Pilla A.A. (1989). Low intensity pulsed ultrasound increases calcium incorporation in both differentiating cartilage and bone cell cultures. Transactions of the Annual Meeting : *Orthopaedic Research Society* 14, 15.

Sousa V.L., Alvarenga J., Padilha Filho J.G., Canola J.C., Ferrigno C.R.A., Alves J.M., Duarte L.R. (2008). Low-intensity pulsed ultrasound in diaphyseal fractures: clinical application in dogs. *Ciencia Rural* 38 (4), 1030-1037.

Steiss J.E. (2003). Canine rehabilitation. In: Braund K.G. (editor). Clinical neurology in small animals – Localization, diagnosis and treatment, www.ivis.org .

Tanzer M., Harvey E., Kay A., Morton P., Bobyn J.D. (1996). Effect of noninvasive low intensity ultrasound on bone growth into porous-coated implants. *Journal of Orthopaedic Research* 14 (6), 901-906.

Tajali S., Houghton P., MacDermid J.C., Grewal R. (2012). Effects of low-intensity pulsed ultrasound therapy on fracture healing: A systematic review and meta-analysis. *American Journal of Physical Medicine & Rehabilitation* 91(4), 349-367.

Ter Haar G. (2007). Therapeutic applications of ultrasound. *Progress in Biophysics and Molecular Biology* 93, 111- 129.

Wang E.D. (1998). Tendon repair. *Journal of Hand Therapy* 11(2), 105-110.

Wang C-J., Chen H-S. en Yang D. (2001). Treatments of nonunions of long bone fractures with shock waves. *Clinical Orthopaedics and related research* 387, 95-101.

Watson T. (2006). Electrotherapy and tissue repair. *Sportex-Medicine* 29, 1-13.

Watson T. (2008). Ultrasound in contemporary physiotherapy practice. *Ultrasonics* 48, 321-329.

Welgus H.G., Jeffrey J.J., Eisen A.Z. (1981). Human skin fibroblast collagenase. Assessment of activation energy and deuterium isotope effect with collagenous substrates. *Journal of Biological Chemistry 256, 9516-9521*.

Wood R.W., Loomis A.L. (1927). The physical and biological effects of high frequency sound waves of great intensity. *Philosophical Magazine* 4, 417-436.

Wu C.C., Lewallen D.G., Bolander M.E., Bronk J., Kinnick R., Greenleaf J.F. (1996). Exposure to low intensity ultrasound stimulates aggrecan gene expression by culture chondrocytes. Transactions of the Annual Meeting: Orthopaedic Research Society 21, 622.

Yang K.H., Park SJ. (2001). Stimulation of fracture healing in a canine ulna full-defect model by low-intensity pulsed ultrasound. *Yonsei Medical Journal* 42 (5), 503-508.

Young S.R., Dyson M. (1990). The effect of therapeutic ultrasound on angiogenesis. *Ultrasound in Medicine and Biology* 16, 261-269.

Exercises in canine physical rehabilitation: range of motion of the forelimb during stair and ramp ascent

Jennifer Carr[1], Darryl. L. Millis and Hsin-Yi Weng

SUMMARY

Objectives: To evaluate overall joint range of motion of the forelimb in healthy dogs ascending stairs compared with incline slope walking.

Methods: Normal canine forelimb kinematics (range of motion, flexion and extension) were compared during ascent of stairs or a ramp, and compared to unimpeded trotting on a flat surface. Eight adult dogs with no evidence of orthopaedic or neurological lameness were assessed using a 2-dimensional kinematic system as they walked up a custom built ramp and stairs.

Results: In healthy dogs, ramp and stair ascent consistently had greater range of motion compared to trotting on a flat surface, and ramp ascent had significantly greater range of motion compared to stair ascent (P<0.05). Shoulder flexion and extension, elbow extension and carpal flexion were all significantly greater while ascending the ramp compared to stairs. Shoulder extension on the flat was significantly greater than while ascending stairs.

Clinical Significance: When planning physical rehabilitation exercises following injury to the forelimb, stair and ramp ascent may be considered, as both augment range of motion of joints. Ramp ascent provides the greatest increase in range of motion of fore-limb joints.

Introduction

Forelimb injuries, and neurological and orthopaedic conditions are common sources of gait abnormalities in dogs. Successful recovery from neurological or orthopaedic injury to the forelimb may be enhanced with the use of physical rehabilitation (Canapp et al. 2009, Davidson et al. 2005, Hamilton 2004). The goal of physical rehabilitation

is to improve or maintain function, while reducing pain. Increasing overall joint range of motion (ROM) of affected joints may require the use of activities other than walking or trotting on a level surface (Marsolais et al. 2003). Recovery from many of these injuries, such as shoulder and elbow luxation, may benefit from active and passive ROM (Davidson et al. 2005). Use of inclined surfaces and stairs for forelimb injuries has been described, but little research has been done to evaluate the effects of each.

ROM is crucial in order to enhance or maintain joint function (Hamilton 2004, Saunders et al. 2005). In addition to primary conditions of the forelimb, joints of the forelimb may also compensate for conditions affecting the hindlimb because of weight shifting from the pelvic limbs to the forelimbs. This may lead to chronic forelimb

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lameness over time. Thus, activities that affect ROM are of particular importance to the physical rehabilitation practitioner.

Kinematic evaluation provides an objective way of measuring motion by describing joint angles, velocity, acceleration and stride length. Several systems exist to collect 2- or 3-dimensional (2D, 3D) data. In humans, the use of 3D analysis has been recognised as the standard method of gait analysis (Kim et al. 2008). However, a recent study evaluating the difference between the two systems suggests that 2D systems can provide accurate and repeatable data when analysing canine pelvic limbs in the sagittal plane at a walk (Kim et al. 2008, Feeney et al. 2007). Joint angle changes from a 2D system can be reported in various planes, such as linear, coronal and diagonal (Durant et al. 2011, Kim et al. 2008), however, measurements in the transverse and frontal planes have not been studied extensively. Thus, output from either 2D or 3D systems can be used to evaluate joint ROM in dogs.

ROM in joints of the forelimb has not been evaluated as thoroughly as the hindlimb in dogs. A recent study evaluated normal forelimb walking on a level surface compared to uphill and downhill walking on a treadmill, and to low cavaletti rails (Holler et al. 2010). They concluded that walking on a treadmill at a grade or incline of 11% (angle of inclination 6.3°) had no significant effect on the forelimb joint ROM when compared to walking on a level surface. However, it is possible that altering the incline could result in a significant change in ROM. Perhaps by using a steeper slope, or, by using stairs instead of a flat ramp, ROM could be increased compared to activity over level surfaces. In dogs, kinematic analysis has been used to describe ROM of the hindlimb in normal and in cranial cruciate deficient stifles (Marsolais et al. 2003), as well as in dogs with hip dysplasia (Bockstahler et al. 2007) and elbow disease (Burton et al. 2008). It has also been used to describe ROM of pelvic limbs of normal dogs during different activities such as swimming (Marsolais et al. 2003), and descent of stairs and ramps (Durant et al. 2011, Millard et al. 2010).

When reviewing data concerning inclined surfaces, it is important to clarify definitions pertaining to the incline. The slope is also the tangent of the angle of elevation. The inverse tangent of the grade, or percent inclination as given by a treadmill, will be the angle of elevation. Therefore, the terms grade and percent inclination can be

used interchangeably, but are not the same as slope or the angle of elevation.

Recently, Durant et al. (2011) evaluated motion of the major joints of the pelvic limb during ascent of stairs and ramp compared to a flat surface. They concluded that dogs undergo greater hindlimb ROM while ascending stairs compared to a ramp and flat surface. However, the ramp angle was not as steep as the stairs. This suggests that steeper angles of incline may be required to significantly change the ROM in the pelvic limb from that on a flat surface or stairs, and similar factors may exist regarding exercises for forelimb motion. This information may be useful, particularly because stair exercises are commonly recommended in dogs for rehabilitation (Durant et al. 2011). However, it is important to realise there are significant differences in forelimb and hindlimb weight distribution. It is a well-accepted fact that the forelimbs bear roughly 60% of a dog's individual bodyweight, whereas the hindlimbs bear 40% during walking on a flat surface (Budsberg et al. 1987). At a minimum, bodyweight and conformation make significant contributions to weight distribution in individual dogs (Voss et al. 2011). Information regarding forelimb weight distribution and motion is sparse and may be beneficial in implementing clinical activities to enhance or maintain ROM of the forelimb following injury in dogs.

The purpose of the study reported here was to evaluate the shoulder, elbow and carpal joints of normal dogs during ascent of stairs and a ramp of equal inclination, and trotting on a level surface. It was hypothesised that there would be a significant increase in ROM with ascent of both inclined surfaces as compared to trotting on a flat surface, and that forelimb motion would not be significantly different between ascent of an inclined ramp or stairs.

Materials and methods

Eight female adult hound-type mixed breed dogs were included in the study. Bodyweight ranged from 21.3 to 24.5 kg. At the time of the study, all dogs were approximately five years of age. All dogs were healthy as determined by haematologic and clinical chemistry data. In addition, all dogs were deemed sound by thorough physical, orthopaedic and neurological examinations before the study. The study was approved by the University of Tennessee's animal care and use committee.
Forelimb joint motion in the sagittal plane was assessed

while jogging and during ascent up a set of standard stairs and a ramp (Fig 1). The stairs had an angle of inclination of 35° (70% grade). The dimensions for each of the four steps were height 17.78 cm, length 25.4 cm and width 91.4 cm. The stairs served as the base for the ramp, which therefore also had a mean angle of inclination of 35°s (70% grade). The ramp was fashioned by covering the staircase with a platform and commercial-grade carpet. Dogs were accustomed to ascent of the stairs and ramp before entering the study. The same handler was used for all trials.
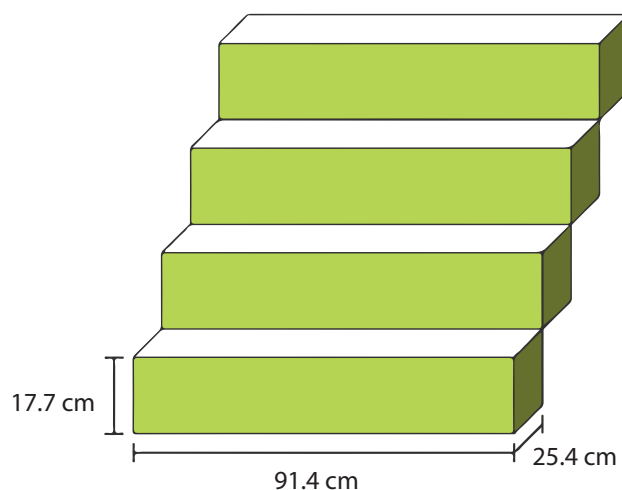

17.7 cm

91.4 cm

25.4 cm

FIG 1. Replication of custom stairs with individual step dimensions of 25.4 cm×91.4 cm×17.8 cm. (reprinted with permission from Durant et al. 2011)

Kinematic analysis was performed using a 2D digital capture system (Peak Motus). Hair was clipped and dogs were outfitted with spherical, reflective markers (1.3 cm in diameter) on the skin overlying the proximal spine of the scapula, greater tubercle of the humerus, lateral epicondyle of the humerus, ulnar carpal bone and the head of the fifth metacarpal bone. Digital, infrared cameras (Phillips Electronics) were arranged in a semi-circular pattern approximately 4 m from the recording area. The system was calibrated at the beginning of each study using a standard calibration wand.

Successful trials included those in which the dog ascended the ramp or staircase at a natural walking pace. Five successful trials of stair and ramp ascent were recorded. For each joint, extension angles, flexion angles and overall joint ROM were determined in the sagittal plane. ROM was equal to the difference between maximum joint extension and maximum joint flexion during each trial.

Trotting data were subsequently collected over a flat surface. Acceptable velocity was between 1.7-2.1 m/s and acceleration or deceleration was less than or equal to

$\pm 0.5 m/s^2$. Five trials were obtained and averaged for each full gait cycle.

Data were assessed for normality using the Shapiro–Wilk test. All variables were normally distributed; therefore mean and standard deviation (sd) were reported. For each joint, ROM, flexion and extension angles were compared among stairs, ramp and a flat surface using repeated measures ANOVA followed by pairwise comparisons between surfaces. Ninety-five percent confidence intervals were plotted to quantify the uncertainty of estimation. Statistical significance was defined as $P < 0.05$.

Results

Shoulder

There was a significant difference in peak flexion between the ramp (mean 85.3°; sd 20.5°) and stairs (mean 105.8°, sd 10.1°), with greater flexion occurring while ascending the ramp (Fig 2). Flexion while ascending the ramp was significantly greater than while trotting on a flat surface (mean 119.3°, sd 4.6°). Shoulder peak extension on the ramp (mean 151.8°, sd 12.8°) was significantly greater than trotting on a flat surface (mean 137.9°, sd 3.6°). Shoulder extension trotting on a flat surface was significantly greater than ascending stairs (mean 126.0°, sd 8.6°, $P < 0.01$). However, shoulder extension while ascending the ramp was significantly greater than while ascending the stairs and trotting. Shoulder ROM was significantly greater while ascending the ramp (mean 66.5°, sd 13.3°) when compared to stairs (mean 20.2°, sd 5.3°) or trotting on a flat surface (mean 18.6°, sd 2.7°). However, there was no significant difference in ROM between the stairs and flat surface trotting.
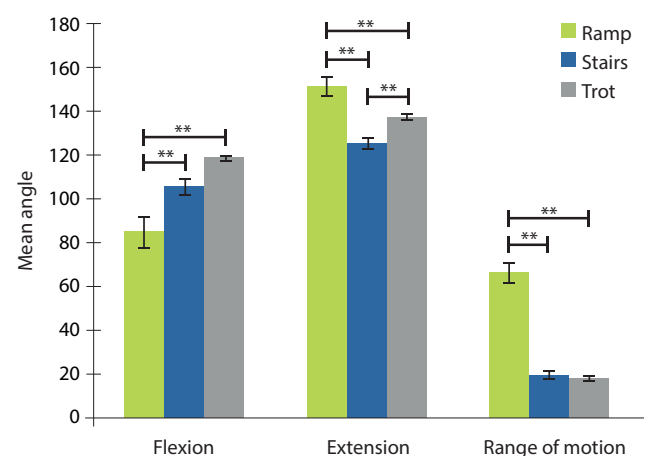

*FIG 2. Graph indicating shoulder flexion, extension and ROM for each condition: **P<0.01, *P<0.05. Error bars are 95% confidence intervals*

Elbow

Elbow flexion while ascending both the ramp and stairs was significantly greater than trotting on the flat (mean 93.6°, sd 10.9°) (Fig 3). Although peak elbow flexion was greater during ascent of the ramp (mean 45.7°, sd 8.4°) compared to stairs (mean 57.0°, sd 8.4°), this difference was not significant. Extension for both the ramp (mean 156.6°, sd 10.8°) and stair ascent were both significantly greater than for trotting (mean 139.6°, sd 6.3°), and elbow extension was significantly greater while ascending the ramp than the stairs (mean 149.0°, sd 5.5°). Elbow ROM was also significantly greater while ascending the ramp (mean 110.9°, sd 13.1°) compared with the stairs (mean 92.0°, sd 7.2°). Ramp and stair values for ROM of the elbow were significantly greater when compared to trotting on a flat surface (mean 46.0°, sd 6.2°).
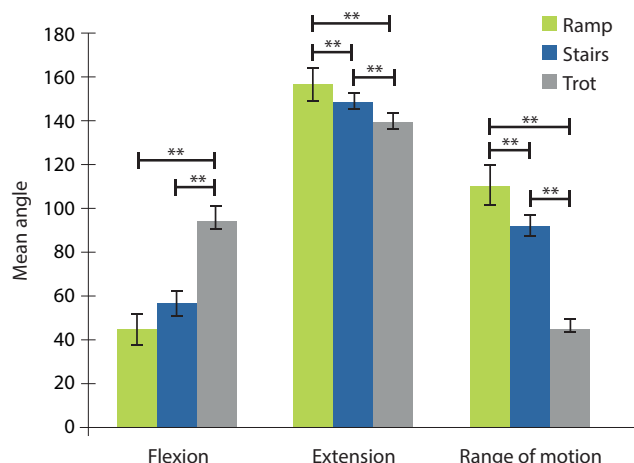

FIG 3. Graph indicating elbow flexion, extension and ROM for each condition: **P<0.01, *P<0.05. Error bars are 95% confidence intervals

Carpus

Carpal flexion was greater while ascending stairs (mean 68.1°, sd 14.0°) and the ramp (mean 35.6°, sd 22°) when compared to trotting on a flat surface (mean 83.9°, sd 23.5°) (Fig 4), and flexion while ascending a ramp was greater when compared to the stairs. Carpal extension was significantly greater for both stair (mean 171.0°, sd 3.1°) and ramp (mean 172.5°, sd 4.1°) ascent when compared to trotting on a flat surface (mean 160.8°, sd 5.8°). However, there were no differences in extension between stair and ramp ascent. Carpal ROM was significantly greater while ascending the ramp (mean 136.9°, sd 20.4°) compared to stairs (mean 102.9°, sd 12.3°). Carpal ROM was significantly greater for both stair and ramp ascent compared to trotting (mean 76.9°, sd 24.0°).
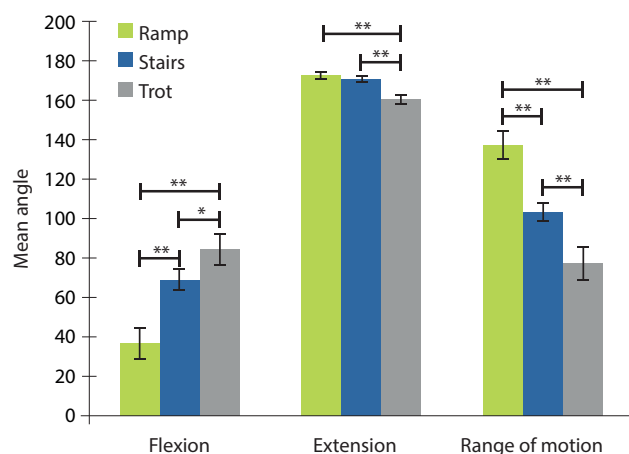
FIG 4. Graph indicating carpal flexion, extension and ROM for each condition: **P<0.01, *P<0.05. Error bars are 95% confidence intervals

Discussion

The results of this study indicate that normal dogs achieve greater overall forelimb joint ROM while ascending stairs and ramps compared to trotting on a flat surface. In addition, ROM was greater while ascending a ramp when compared to stairs. These differences were in large part due to significant increases in both flexion and extension of each joint. As anticipated, the most obvious differences were those comparing ramp or stairs to trotting over a flat surface.

The data collected in this study differ from those obtained in a recent study evaluating ROM parameters in the forelimb over flat and inclined surfaces (Holler et al. 2010). In that study, no significant differences were found comparing motion of the forelimb on an incline to a flat surface. An important difference between that study and the present one is the angle of inclination of the ramp. In the referenced study, the angle of inclination of the surface was 6.3°, while the angle of inclination here was 35°. Thus, collectively these studies suggest that an inclined surface greater than 6.3° may result in increased forelimb joint angles. However, it is not clear at which angle beyond that the forelimb joint angles increase significantly.

Another possible reason for the discrepancy is related to the study dogs. In the referenced study, eight client-owned dogs of different breeds with a bodyweight of 23.6 ±4.6 kg were enrolled. The dogs in this report were eight hound-type dogs weighing 22.13 ±1.41 kg. Other workers have suggested that when collecting kinetic data, dogs of different breeds may not be comparable to each other and that group comparisons should not be made among dogs of

different bodyweight and conformation (Voss et al. 2011). It is also possible that even dogs of the same breed, but of significantly different size or bodyweight would be different. It is not known what the effect of bodyweight and body conformation on kinematic variables is, but it seems plausible that changes in joint angles during gait are a function of body conformation.

The reason for the increased motion when ascending the ramp compared to stairs is unclear. A recent study evaluated the difference in pelvic limb joint motion while descending a ramp and continuous slope (Millard et al. 2010). The authors concluded that pelvic limb joints of normal dogs achieved different ROM during descent of stairs and an equivalent ramp. In contrast to the results presented here, they found that greater active ROM was achieved in each joint of the pelvic limb during stair descent. The authors speculated that the reason for such differences in ROM is related to the dogs' stride length. While walking down a ramp, dogs can adjust their stride length as compared with travelling down stairs, which requires moving the limb a fixed distance. Although stride length was not measured in this study, the same speculations regarding stride length changes during stair and ramp descent can be made regarding uphill climbing of stairs and a ramp. Ascending a ramp may allow for an extended ROM of all joints, but in particular the shoulder, because of less restriction of stride length.

Forelimb lameness, especially diseases of the shoulder and elbow, are common and often benefit from physical rehabilitation following injury or surgery (Davidson et al. 2005). Depending on the nature of the injury, it may be advisable to either limit or enhance ROM. For example, a common injury of sporting dogs is sprain of the medial glenohumeral ligament, for which surgical treatment may not be an option (Marcellin-Little et al. 2007). Early rehabilitation for this condition may include rest of the affected joint. However, ROM exercises are ultimately required to restore the joint to normal function. The data of this study may provide useful information to guide the development of physical rehabilitation protocols for various conditions, which may benefit from undergoing activities that aim to increase or maintain ROM.

Stairs and inclined surfaces have not only been employed to increase the ROM of joints they also have been used as methods of strengthening the pelvic limb muscles after injury or disuse (Millis 2004). Although this study was not designed to identify challenged muscle groups, one can speculate that certain muscle groups are strengthened when ascending stairs or inclined surfaces. In a study by Durant et al. (2011), it was speculated that an increased ROM may have an effect on certain muscles of the pelvic limb while ascending stairs. The same may be true of the forelimb, whereby the main extensors of the shoulder and elbow have the greatest influence on ROM. However, future research in this area is warranted.

There were several limitations to this study. The small and homogeneous sample size used may make translation of the information to dogs of different bodyweight and conformation, as well as dogs with lameness of the forelimb, difficult. It is possible that ROM would be different in breeds of different build or size (Kim et al. 2011). One could speculate that results may be more variable with stairs as compared with the ramp, because of the discrete distance of the step requiring greater relative limb excursion in small breeds as compared with giant breeds. A recent study speculated that with a ramp, dogs can adjust their stride length with decline walking, whereas they must move the limb a fixed distance when negotiating stairs (Millard et al. 2010). Similarly, the dogs in this study may have made adjustments to stride length while ascending the ramp. Proportionally, stride length and joint motion would likely be similar among breeds of different sizes while ascending a ramp of equal slope. Finally, this study evaluated only one angle of inclination. It may be worthwhile to compare several different inclinations in one study to evaluate the impact of variations in angle. It is possible that there is a certain cutoff, high or low, above or below which changes in inclination have no further effect on joint motion.

Recommendations cannot be made on the exact inclination of ramp incline that would be clinically useful for physical rehabilitation of dogs. However, the angle of inclination of the stairs was similar to that found in an average household. It is known that changes in weight shift occur in the forelimbs during activities that change the centre of gravity (Voss et al. 2011). Propulsion from the hindlimbs is the predominant force during incline ascent and braking of the forelimbs is predominant in stair descent. Therefore, evaluating forelimb ROM during stair and incline descent is also warranted.

Future studies are warranted to evaluate the usefulness of these activities in dogs with conditions of the forelimb.

The differences in ROM may be less when motion is limited because of disease or pain, especially for ascending a ramp where an individual could adjust the stride length if discomfort exists. It would be helpful to determine the exact angle of inclination required to significantly increase ROM.

On the basis of the results of the study reported here, it is evident that ramp and stair ascent may elicit greater ROM of the joints in the forelimb as compared to trotting over ground. This information may be useful when developing physical rehabilitation protocols following injury or surgery to the forelimb. This information may be used in the design of physical rehabilitation protocols that are focused on maintenance or enhancement of joint angles.

References

Bockstahler, B. A., Henninger, W., Müller, M., et al. (2007) Influence of borderline hip dysplasia on joint kinematics of clinically sound Belgian Shepherd dogs. *American Journal of Veterinary Research* 68, 271-276

Budsberg, S. C., Verstraete, M. C. & Soutas-Little, R. W. (1987) Force plate analysis of the walking gait in healthy dogs. *American Journal of Veterinary Research* 48, 915-918

Burton, N. J., Dobney J. A., Owen, M. R., et al. (2008) Joint angle, moment and power compensations in dogs with fragmented medial coronoid process. *Veterinary and Comparative Orthopaedics and Traumatology* 21, 110-118

Canapp, S., Acciani, D., Hulse, D., et al. (2009). Rehabilitation therapy for elbow disorders in dogs. *Veterinary Surgery* 38, 301-307

Davidson, J. R., Kerwin, S. C., Millis, D. L. (2005). Rehabilitation for the orthopedic patient. The Veterinary Clinics of North America; *Small Animal Practice* 35, 1357-1388

Durant, A. M., Millis, D. L. & Headrick, J. F. (2011). Kinematics of stair ascent in healthy dogs. *Veterinary and Comparative Orthopaedics and Traumatology* 24, 99-105

Feeney, L. C., Lin, C. F., Marcellin-Little, D. J., et al. (2007). Validation of two-dimensional kinematic analysis of walk and sit-to-stand motions in dogs. *American Journal of Veterinary Research* 68, 277-282

Hamilton, S. (2004). Therapeutic exercises. In: Canine Rehabilitation and Physical Therapy. 1st edn Eds D. L. Millis and D. Levine. W. B. Saunders, Philadelphia, PA, USA. pp 244-263

Holler, P. J., Brazda, V., Dal-Bianco, B., et al. (2010). Kinematic motion analysis of the joints of the forelimbs and hind limbs of dogs during walking exercise regimens. *American Journal of Veterinary Research* 71, 734-740

Kim, J., Rietdyk, S. & Breur, G. J. (2008). Comparison of two-dimensional and three-dimensional systems for kinematic analysis of the sagittal motion of canine hind limbs during walking. *American Journal of Veterinary Research* 69, 1116-1122

Kim, J., Kazmierczak, K. A. & Breur, G. J. (2011). Comparison of temporospatial and kinetic variables of walking in small and large dogs on a pressure-sensing walkway. *American Journal of Veterinary Research* 72, 1171-1177

Marcellin-Little, D. J., Levine, D. & Taylor, R. (2005). Rehabilitation and conditioning of sporting dogs. The Veterinary Clinics of North America; *Small Animal Practice* 35, 1427-1439

Marsolais, G. S., McLean, S., Derrick, T., et al. (2003). Kinematic analysis of the hind limb during swimming and walking in healthy dogs and dogs with surgically corrected cranial cruciate ligament rupture. *Journal of the American Veterinary Medical Association* 222, 739-743

Millard, R. P., Headrick, J. F. & Millis, D. L. (2010). Kinematic analysis of the pelvic limbs of healthy dogs during stair and decline slope walking. *The Journal of Small Animal Practice* 51, 419-422

Millis, D. L. (2004). Getting the dog moving after surgery. *Journal of the American Animal Hospital Association* 40, 429-436

Saunders, D. G., Walker, J. R. & Levine, D. (2005). Joint mobilization. *The Veterinary Clinics of North America; Small Animal Practice* 35, 1287–1316

Voss, K., Wiestner, T., Galeandro, L., et al. (2011). Effect of dog breed and body conformation on vertical ground reaction forces, impulses, and stance times. *Veterinary and Comparative Orthopaedics and Traumatology* 24, 106-112

Changes in systolic blood pressure over time in healthy cats and cats with chronic kidney disease

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SUMMARY

Background: Hypertension is a common problem in older cats, most often associated with chronic kidney disease (CKD). Cross-sectional studies have suggested that blood pressure in cats increases with age.

Hypothesis/Objectives: To determine whether blood pressure in cats increases with age and whether this occurs independently of the presence of CKD. To investigate risk factors for developing hypertension.

Animals/Subjects: Two hundred and sixty-five cats with CKD and 133 healthy cats ≥9 years were retrospectively identified.

Methods: Four groups were created according to status at initial evaluation (CKD or healthy) and blood pressure at the last included visit (normotensive [NT] or developed hypertension [DH]): Healthy-NT, Healthy-DH, CKD-NT and CKD-DH. Systolic blood pressure (SBP) over time slopes were compared with 0 and between groups. Risk factors for the development of hypertension were investigated, and associations of biochemical and clinical variables with SBP were examined.

Results: Cats that were hypertensive at CKD diagnosis (n = 105) were not included in further analyses. Twenty-seven cats with CKD and 9 healthy cats developed hypertension ≥3 months after diagnosis of CKD or their first visit. Systolic blood pressure significantly increased with age in all cats (P < 0.001). Healthy cats were at less risk than cats with CKD to become hypertensive (hazard ratio 0.2, P < 0.001), with creatinine being an independent risk factor for the development of hypertension.

Conclusions and Clinical Importance: The high prevalence of hypertension in azotemic cats in this study shows the importance of monitoring of SBP in elderly cats, and in particular in cats with CKD.

Key words: Feline; Hypertension; Renal disease; Risk factors.

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Abbreviations:

BPM	beats per minute
CKD	chronic kidney disease
DH	developed hypertension
IQR	interquartile range
NT	remained normotensive
PCV	packed cell volume
RAAS	renin-angiotensin-aldosterone system
SBP	systolic blood pressure
USG	urine specific gravity

Hypertension is a common problem in older cats but, in contrast to humans, where 95–99% cases of hypertension are considered to be primary or essential hypertension, hypertension in cats is most often associated with underlying diseases such as chronic kidney disease (CKD).[1] Increased serum creatinine concentrations occur in up to 74% of hypertensive cats and 19–65% of cats with CKD are hypertensive.[2–5] In humans, the prevalence of CKD increases with increasing age, and the same is true for cats.[6,7] Mean values of systolic blood pressure (SBP) also increase with age in most human populations, as does the prevalence of clinical hypertension.[8,9] In cats, it has been suggested that age is a predisposing factor for the development of hypertension. A cross-sectional study grouping healthy cats into three age groups (<5, 5–10 and older than 10 years) showed higher SBP in the older age groups, although renal function was not assessed in all cats that were classified as healthy.[10] There is a positive correlation between age and mean arterial pressure, but the age distribution of the cats included in this study is not known, which precludes interpretation of the results.[11] Chronic kidney disease and hypertension are both common in older cats and current consensus is that hypertension is secondary to CKD, although the possibility still exists that the hypertension occurs independently of the presence of CKD or actually causes the renal injury.[12] The question can be raised whether this increase in prevalence of hypertension and CKD in older cats is purely age related, or whether the development of hypertension in most cats is secondary to an inability of the kidney to regulate blood pressure.[12] Investigations into the risk factors for blood pressure to increase in both healthy older cats and cats with CKD may aid in answering this question. The hypothesis of this study is that the blood pressure increases with age in senior cats and that the rate of

this increase, and therefore the occurrence of clinically significant hypertension, is greater in cats with CKD.

Materials and Methods

Case Selection

Cats that were 9 years of age or older and examined for the first time at two first opinion practices in central London (People's Dispensary for Sick Animals in Bow and Beaumont Sainsbury's Animal Hospital in Camden) between August 2000 and August 2012 were retrospectively identified. All cats had a follow-up period of ≥3 months and had visited one of the two veterinary practices a minimum of three times. On every visit, a full history was obtained and a physical examination was performed. Systolic blood pressure was measured at all visits using a noninvasive Doppler technique[a] after a period of acclimatization and the SBP was calculated as the average of five consecutive readings. Indirect fundoscopy after applying one drop of tropicamide 1% to both eyes was performed at the end of the consultation if average SBP was ≥160 mmHg. At recruitment into the study, the owner consented to collection of blood samples via jugular venipuncture and urine samples by cystocentesis. Blood samples were collected into lithium heparin and held on ice (4°C) for a maximum of 6 hours before centrifugation[b] and separation. Plasma biochemistry was performed at an external laboratory[c]. Total thyroxine concentration was assessed in all cats that showed clinical signs of hyperthyroidism (e.g. polyphagia, weight loss, tachycardia, and palpable goiter) or had plasma biochemical findings that raised concern (increased alanine aminotransferase or alkaline phosphatase activity). If the bladder was palpable and a urine sample could be collected, a dipstick evaluation was performed and a urine specific gravity (USG) was measured in all cats. In addition to this, the sediment of samples was evaluated microscopically and, if bacteria or an active sediment was found, urine was sent for bacterial culture and sensitivity testing. All cats diagnosed with hyperthyroidism (total T4 > 55 nmol/ L) were excluded from the study, unless a thyroidectomy had been performed ≥90 days before inclusion, to allow stabilization of glomerular filtration rate.[13] Visits from cats that were being treated with glucocorticoids, nonsteroidal anti-inflammatory drugs, antihypertensive medications, erythropoietin, or intravenous fluid therapy were excluded. For the purposes of this study, systemic hypertension was diagnosed when cats had a SBP ≥170 mmHg on two consecutive visits (reliably placing them in the category that

is at moderate risk to develop target organ damage)[12] or SBP ≥160 mmHg with concurrent evidence of hypertensive retinopathy or choroidopathy on indirect fundoscopy. All hypertensive cats were started on amlodipine at a dose of 0.625 mg once daily at the time of diagnosis. CKD was diagnosed in cats with renal azotemia (plasma creatinine concentration ≥2.0 mg/dL either on two consecutive visits or in conjunction with USG <1.035). All owners of cats diagnosed with CKD were advised to feed their cat a commercially available renal diet. Cats were considered healthy if no significant clinical signs were reported by the owner, no abnormalities were found on clinical exam, and the blood results showed no evidence of CKD or other pathologies. Cats were classified according to the diagnosis of CKD and their SBP; the CKD-normotensive group (CKD-NT-group) was composed of cats diagnosed with CKD that did not meet the hypertensive criteria during follow-up, cats in the CKD-developed-hypertension group (CKD-DH-group) developed hypertension ≥3 months after diagnosis of CKD, cats that met the criteria for being healthy were included in the healthy-normotensive group (Healthy-NT-group), and cats were included in the healthy-developed-hypertension group (Healthy- DH-group) if the criteria for being healthy were met, but the cat was diagnosed with idiopathic hypertension during follow-up of ≥3 months. Re-examination of cats diagnosed with CKD was offered at a maximum of 8 week intervals and blood- and urine samples were obtained at a maximum of 16 week intervals. Re-examination of healthy cats was offered at a maximum of 6 month intervals, at which point blood and urine samples were obtained. If a healthy cat was diagnosed with CKD, its penultimate visit before developing azotemia was excluded from the analysis as it is likely that nonazotemic CKD was already present at that stage.

Statistical Analyses

All statistical analyses were performed using commercially available softwared statistical analysis. P-values ≤0.05 were considered significant. Descriptive statistics are presented to define the population, SBP, biochemical and clinical variables at the first and last visit, and are presented as median and interquartile range (IQR). Comparisons between groups were performed using a nonparametric Kruskall-Wallis or parametric ANOVA with post-hoc comparisons where appropriate. Continuous variables were graphically assessed for normality and values were log transformed if normality criteria were not met to allow use in the linear mixed effects model and the time-dependent Cox proportional hazards model. Systolic

blood pressure at the first visit between all four groups was compared using a Kruskal–Wallis test with a Bonferroni adjusted post-hoc comparison. The rates of change in SBP over time and associations between SBP and biochemical (sodium, chloride, phosphate, total calcium, potassium, creatinine, packed cell volume (PCV), albumin, cholesterol) and clinical (weight, heart rate) variables over time were compared between all groups using a linear mixed effects model with subjects as random effects, and time, group and sex as fixed effects. In the CKDDH-group and Healthy-DH-group, the actual SBP measurement that resulted in the diagnosis of hypertension was not included in the analysis. In the CKD-NT-group and Healthy-NT-group all available visits were included. Not all cats had all information available at all visits as, in general, blood samples were taken every other visit, but having a SBP measurement was a requirement for the visit to be included in the models. Change in SBP over time is expressed as ΔmmHg/100 days. A time-dependent Cox proportional hazards model and Kaplan–Meier curves were used to assess the association of the biochemical and clinical variables with the risk of becoming hypertensive. The assumption of proportional hazards was checked for all variables included in the model and cases were censored if they died, were lost to follow-up (defined as not seen for >6 months and not contactable by telephone), or if the study end point was reached. To investigate the independent association of the significant variables with SBP (linear mixed model) and the risk of becoming hypertensive (time dependent Cox proportional hazards model), all significant variables at the P < 0.10 level were included in a multivariable analysis.

Results

A total of 265 cats were diagnosed with azotemic CKD between August 2000 and August 2012 and were seen for three or more visits, over the course of more than three months. Of these cats, 105 were hypertensive at diagnosis of CKD or were diagnosed with systemic hypertension within 3 months of CKD diagnosis. These cats were excluded from further analyses. Of the remaining 160 cats with CKD, 133 (83%) remained normotensive 856 Bijsmans et al throughout follow-up (CKD-NT group), and 27 cats of the 160 cats that were initially normotensive (17%) developed hypertension ≥3 months after CKD diagnosis (CKD-DH group). A total of 133 cats were included as healthy cats in this study of which 9 (7%) developed hypertension (Healthy-DH group), and 124 healthy cats remained normotensive throughout follow-up (Healthy- NT group).

Demographic Data

An overview of the clinical and biochemical variables at baseline and at the last visit (NT-groups: last included visit; DH-groups: hypertensive visit) is presented in Tables 1 and 2. The majority of cats included in the four groups were domestic shorthair (n = 229), followed by domestic longhair (n = 34), and 147 cats were female (of which 4 not neutered) and 146 cats male (of which 4 not neutered). Of the 105 cats that were diagnosed as hypertensive at or within 3 months of CKD diagnosis, 73 (70%) had visible retinal lesions, whereas of the CKD-DH group 52% (14 cats) and of the Healthy-DH group, 3 cats (33%) had evidence of hypertensive retinopathy.

Initial Visit

Chronic kidney disease-cats that remained normotensive during follow-up had significantly lower mean SBP at their first visit than CKD-cats that developed hypertension, and Healthy-NT cats had a significantly lower SBP than Healthy-DH cats (P < 0.05) (Table 1). The SBP at baseline between the NT-groups did not significantly differ, and neither did the DH-groups (Table 1). Cats included in the Healthy-NT group were significantly younger than both groups of cats with CKD (P < 0.001) but the other groups did not significantly differ in age (Table 1).

Rate of Change in Systolic Blood Pressure over Time

The CKD-DH, CKD-NT and Healthy-NT cats all had a significant increase in SBP over time, although the rates of change were not significantly different from each other (P = 0.09; CKD-NT: 0.5 ± 0.1, Healthy-NT: 0.4 ± 0.1 mmHg, CKD-DH: 1.1 ± 0.3, Healthy-DH: 0.3 ± 0.5 mmHg/100 days). All rates of change, aside from the Healthy-DH group, were significantly different from 0 (P < 0.001).

Association Between Systolic Blood Pressure and Biochemical Variables over Time

Using measurements from all time points available for each cat, no significant association between creatinine, sodium, phosphate, total calcium, potassium, and weight and SBP over time could be found. Cholesterol, PCV, albumin and heart rate were significantly and positively associated with SBP and chloride was negatively associated with SBP in the univariable analysis (Table 3). In the multivariable model heart rate (0.07 ± 0.02 mmHg/bpm, P < 0.001) and PCV (0.2 ± 0.1 mmHg/L/L, P < 0.05) remained significantly and positively associated with SBP.

Table 1. Clinicopathologic variables for all groups at initial visit.

Variable	CKD-NT (n = 133)	CKD-DH (n = 27)	Healthy-NT (n = 124)	Healthy-DH (n = 9)
Age (years)	14 (12, 16)[a]	14 (13, 15)[a]	12 (10, 13)[b]	11 (10, 14)[ab]
Weight (kg)	3.9 (3.3, 4.7)[a]	3.7 (3.4, 4.7)[ab]	4.6 (3.6, 5.3)[b]	4.7 (3.9, 5.8)[ab]
SBP (mmHg)	133.2 (121.2, 144.6)[a]	147.2 (140.4, 156.1)[b]	131.6 (115.0, 143.7)[c]	145.6 (139.5, 154)[ab]
Heart rate (bpm)	186 (165, 204)	184 (164, 204)	180 (168, 197)	180 (176, 190)
PCV (%)	34 (32, 38)[a]	36 (31, 39)[a]	39 (36, 41)[b]	36 [34, 38][ab]
Albumin (g/dL)	3.1 (3.0, 3.3)[a]	3.1 (2.9, 3.2)[ab]	3.2 (3.1, 3.4)[b]	3.3 (2.9, 3.5)[ab]
Creatinine (mg/dL)	2.4 (2.2, 2.9)[a]	2.5 (2.3, 2.8)[a]	1.5 (1.4, 1.7)[b]	1.5 (1.5, 1.6)[b]
Urea (mmol/L)	16.9 (14.4, 20.2)[a]	17.0 (13.6, 20.0)[a]	9.9 (8.9, 11.2)[b]	9.5 (8.4, 10.5)[b]
Phosphate (mg/dL)	4.22 (3.63, 5.12)[a]	3.97 (3.43, 4.73)[ab]	3.84 (3.3, 4.73)[b]	4.12 (3.47, 4.46)[ab]
Total calcium (mg/dL)	10.2 (9.9, 10.4)[a]	9.9 (9.7, 10.6)[ab]	9.8 (9.5, 10.3)[b]	10.1 (9.8, 10.6)[ab]
Sodium (mEq/L)	153.0 (151.3, 154.8)[a]	151.7 (150.5, 152.5)[a]	152.4 (151.3, 153.7)[a]	151.0 (150.0, 152.5)[b]
Potassium (mEq/L)	4.10 (3.80, 4.40)	4.10 (3.90, 4.38)	3.90 (3.70, 4.20)	3.80 (3.60, 4.25)
Chloride (mEq/L)	117.8 (116.3, 120.2)[ab]	116.0 (114.8, 118.9)[a]	119.3 (117.2, 120.6)[b]	120.4 (116.5, 121.2)[ab]
Cholesterol (mg/dL)	200 (154, 239)	201 (160, 234)	178 (151, 212)	166 (154, 205)
USG	1.020 (1.017, 1.024)[a]	1.017 (1.016, 1.022)[a]	1.050 (1.038, 1.060)[b]	1.042 (1.039, 1.050)[b]

CKD-DH CKD-developed-hypertension; **CKD-NT** CKD-remained normotensive; **SBP** systolic blood pressure; **PCV** packed cell volume; **USG** urine specific gravity.
Parameters shown are at first visit for all cats. Superscript letters identify groups that differed significantly.

Table 2. Clinicopathologic variables for all groups at last visit.

Variable	CKD-NT (n = 133)	CKD-DH (n = 27)	Healthy-NT (n = 124)	Healthy-DH (n = 9)
Follow-up (days)	316 (225, 512)	379 (281, 771)	666 (495, 1065)	939 (685, 1217)
Age (years)	15 (13, 17)[a]	15 (14, 18)[a]	14 (12, 16)[b]	15 (14, 15)[ab]
Weight (kg)	3.6 (3.0, 4.5)[a]	3.7 (3.2, 4.3)[ab]	4.3 (3.4, 5.1)[b]	4.7 (3.4, 5.7)[b]
SBP (mmHg)	138.4 (120.5, 148)[a]	181.6 (175.0, 188.8)[b]	133.4 (121.1, 146.1)[a]	181.2 (174.8, 190)[b]
Heart rate (bpm)	186 (168, 210)	180 (160, 204)	184 (176, 204)	192 (186, 208)
PCV (%)	34 (30, 38)[a]	36 (29, 39)[ab]	37 (34, 40)[b]	37 (36, 40)[ab]
Albumin (g/dL)	3.1 (2.9, 3.3)	3.1 (2.9, 3.3)	3.1 (3.0, 3.4)	3.2 (3.1, 3.4)
Creatinine (mg/dL)	2.5 (2.1, 3.3)[a]	2.7 (2.4, 3.0)[a]	1.4 (1.3, 1.7)[b]	1.6 (1.4, 1.9)[b]
Urea (mmol/L)	16.9 913.6, 22.0)[a]	17.1 (15.2, 21.1)[a]	10.5 (8.8, 12)[b]	10.9 (9, 11.8)[b]
Phosphate (mg/dL)	4.15 (3.55, 5.04)[a]	4.28 (3.72, 5.16)[a]	3.81 (3.50, 4.31)[b]	4.06 (3.53, 4.56)[ab]
Total calcium (mg/dL)	10.4 (10.0, 10.8)[a]	10.6 (10.1, 11.0)[a]	9.9 (9.5, 10.2)[b]	9.9 (9.8, 10.2)[ab]
Sodium (mEq/L)	154.3 (152.2, 156.4)[a]	153.8 (151.7, 154.9)[ab]	153.6 (152.0, 154.9)[b]	152.7 (151.0, 153.6)[ab]
Potassium (mEq/L)	4.20 (3.95, 4.50)[a]	4.10 (3.95, 4.55)[ab]	4.05 (3.80, 4.30)[b]	4.20 (3.79, 4.40)[ab]
Chloride (mEq/L)	119.1 (117.4, 120.8)	117.7 (115.8, 119.6)	118.9 (117.2, 120.8)	118.7 (117.3, 119.4)
Cholesterol (mg/dL)	200 (157, 247)[a]	212 (177, 256)[ab]	177 (147, 219)[b]	201 (151, 236)[ab]
USG	1.018 (1.016, 1.022)[a]	1.018 (1.015, 1.019)[a]	1.047 (1.032, 1.054)[b]	1.034 (1.034, 1.038)[b]

CKD-DH CKD-developed-hypertension; **CKD-NT** CKD-remained normotensive; **SBP** systolic blood pressure; **PCV** packed cell volume; **USG** urine specific gravity.
Parameters shown are at last included visit for the-NT groups and first hypertensive visit for the-DH groups. Superscript letters identify groups that differed significantly.

Table 3. Linear mixed model investigating the association between biochemical and clinical variables and systolic blood pressure over time.

Variable	Estimate ± SE	p-value
Creatinine (mg/dL)	-3.0 ± 2.2	0.17
Sodium (mEq/L)	-0.03 ± 0.1	0.75
Chloride (mEq/L)	**-0.2 ± 0.08**	**0.04**
Phosphate (mg/dL)	0.4 ± 0.4	0.34
Total calcium (mg/dL)	0.6 ± 0.6	0.30
Potassium (mEq/L)	0.6 ± 1.1	0.56
Cholesterol (mg/dL)	**0.02 ± 0.009**	**0.04**
PCV (%)	**0.30 ± 0.10**	**0.001**
Albumin (g/dL)	**4.5 ± 1.8**	**0.013**
Weight (kg)	0.08 ± 0.07	0.27
Heart rate (bpm)	**0.07 ± 0.01**	**<0.001**

PCV, packed cell volume; SE, standard error.
Values depicted in bold were significantly associated with SBP in the univariable analysis and were included in the multivariable linear mixed model. PCV and heart rate remained significantly associated with SBP.
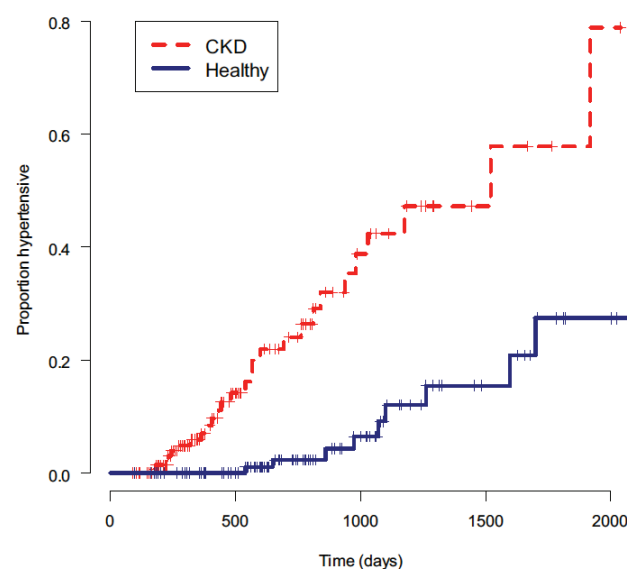

Fig 1. Kaplan–Meier curve of probability to become hypertensive. CKD; chronic kidney disease, time is in days from first visit. Cats with CKD have a greater probability to be hypertensive at each time point than healthy cats (P < 0.001). Censored cases are represented by ticks.

Table 4. Cox proportional hazards model investigating the risk factors for the development of hypertension.

	HR 95%	Confidence interval	P-value
Creatinine (mg/dL)	**9.39**	**2.06–42.82**	**0.004**
Sodium (mEq/L)	1.07	0.90–1.27	0.47
Chloride (mEq/L)	1.00	0.92–1.08	0.93
Phosphate (mg/dL)	**1.34**	**1.03–1.74**	**0.03**
Total calcium (mg/dL)	0.88	0.51–1.53	0.65
Potassium (mEq/L)	0.91	0.28–2.98	0.87
Cholesterol (mg/dL)	1.00	1.00–1.01	0.38
PCV (%)	0.95	0.86–1.05	0.3
Albumin (g/dL)	0.72	0.17–3.11	0.66
Weight (kg)	0.81	0.58–1.14	0.22
Heart rate (bpm)	1.00	0.98–1.00	0.16

PCV, packed cell volume.
Values depicted in bold were a significant risk factor for the development of hypertension in the univariable analysis and included in the multivariable Cox proportional hazards model. Creatinine remained the only independent risk factor for the development of hypertension.

Risk of Becoming Hypertensive

The Cox proportional hazards model showed that at all time points, cats that were healthy were at less risk than cats with CKD of becoming hypertensive (Hazard ratio = 0.2 [95% confidence interval 0.08–0.36], P < 0.001) (Fig 1). Sodium, chloride, total calcium, potassium, cholesterol, PCV, albumin, weight and heart rate were not significant risk factors for the development of hypertension (Table 4). Increased phosphate and creatinine concentrations were found to be significant risk factors for the development of hypertension in the univariable analysis (P < 0.05). Creatinine concentration was the only independent risk factor for a cat becoming hypertensive (Hazard ratio = 7.3 [95% confidence interval 1.2–45.4], P = 0.03).

Discussion

Of the initially normotensive cats with CKD, 17% developed hypertension, whereas of the initially normotensive healthy cats included in this study, 7% did. In addition to this, 105/264 azotemic cats were hypertensive at or within three months of CKD diagnosis. In crosssectional studies, 23% of hypertensive cats are nonazotemic and do not have an identifiable cause for their hypertension,[14] and 12% of nonazotemic, nonhyperthyroid cats are hypertensive

at baseline.[7] This study only included healthy cats that developed hypertension at a later time-point and excluded cats that were presented as an idiopathic hypertensive cat at their first visit to the clinic, which probably explains the lower prevalence. The criteria for defining hypertension and the composition of the study population greatly influence reported prevalence, and the prevalence of hypertension in cats with CKD in the current study is lower than the 65% reported in a study performed in a referral setting,[4] and greater than the 19.4% previously reported in a crosssectional study using a higher SBP cut-off.[3] In addition to this, all cats included in this study had a minimum follow-up of three visits over the course of three months. This could have artificially increased the size of the group of cats that were hypertensive at diagnosis of CKD, as these cats came back for regular blood pressure checks on antihypertensive medication.

Both healthy cats and cats with CKD included in this study showed a significant increase in SBP with increasing age. No significant increase in SBP over time could be found for the healthy-DH group, although this was most likely because of the small group size. The significant increase in blood pressure with age is comparable to the human situation.[15] Studies investigating the age effect on blood pressure in cats are conflicting, but all studies published to date commenting on this phenomenon were cross-sectional in design.[5,10,16–18] A moderate correlation between all blood pressure variables and age has been reported in cats,[18] although a study including only healthy cats did not find a significant correlation between SBP and age.[17] Sansom and co-workers reported a higher blood pressure in older cats if the cats were grouped according to age (younger than 5, 5– 10 years and older than 10). The cats that were included in this study did, however, not all have serum creatinine concentration measured, and the possibility exists that cats with subclinical CKD have been included, especially in the oldest age group.[10] Another study performed in a population of healthy cats, however, could not demonstrate a significant difference between the SBP of cats <10 years of age and cats that were 10 years or older.[5] A further study including healthy and ill cats did report an age cut-off, with cats over the age of 11 years having significantly higher blood pressure than cats younger than 11, although renal function was not assessed in the apparently healthy population of cats.[18] Hypertensive cats have been reported to be significantly older than their normotensive counterparts, although the study population consisted of a mixture of cats including

cats with renal disease and hyperthyroidism.[16] Both diseases are associated with hypertension,[12] and the prevalence of CKD and hyperthyroidism also increases with age,[6,7,19] which means that the age effect found could be due to the inclusion criteria of this study. To our knowledge, this study is the first investigating changes in blood pressures in individual healthy cats and cats with CKD over time and is therefore the first to definitely show by longitudinal analysis that pressure rises with age in cats, as it does in humans.

The positive association between heart rate and SBP has commonly been reported in human literature. Mean arterial pressure is determined by cardiac output and peripheral resistance, and the association between heart rate and SBP and can be attributed to the fact that cardiac output is increased with an increased heart rate.[20] A previous cross-sectional study found no significant correlation between heart rate and SBP in healthy cats.[21] Heart rate has been reported to not be significantly different in cats with higher blood pressures and CKD when compared to healthy cats with lower blood pressures,[11] although a previous study reported 17% of cats with hypertension to be tachycardic.[14] To our knowledge, this study is the first to investigate the association between heart rate and SBP over time in individual cats. A positive independent association between PCV and SBP was found in this study. Hematocrit is the most important determinant of blood viscosity, which in turn determines peripheral resistance together with vascular diameter. Human studies have also described this independent positive association between PCV and SBP, and these studies also report an increased PCV in hypertensive subjects compared to normotensive subjects.[22]

Cats that develop hypertension have a higher baseline blood pressure than their normotensive counterparts. In the human literature the term 'prehypertension' is often used to describe a nonoptimal baseline blood pressure. Blood pressure and the incidence of hypertension increases with age, and human subjects with higher baseline blood pressure are at higher risk of becoming hypertensive.[23,24] The CKD-DH group had a significantly higher blood pressure than both the CKD-NT group and the healthy-NT group. The small size of the healthy-DH group makes interpretation of the result more challenging, although the SBP at time of inclusion in the study seems comparable to the SBP of the CKDDH group. The World Health Organisation-International Society of Hypertension has guidelines to classify nonhypertensive individuals into blood pressure categories (optimum, normal or high-normal). Based on these categories, screening intervals are recommended for human subjects, although these intervals differ between organizations.[24] No optimum recommendations exist yet for screening intervals for individual elderly normotensive cats, but based on the results obtained in this study it could be argued that more frequent blood pressure measurements need to be encouraged if an owner presents a cat with a mean SBP ≥140 mmHg. Cats with CKD have a significantly greater probability of becoming hypertensive, and creatinine is the only independent predictor for becoming hypertensive, which could call for an even shorter interval between screening visits for cats with CKD. Of the cats that were diagnosed as hypertensive, a greater percentage showed signs of target organ damage if they were diagnosed with CKD and hypertension around the same time point (70%), when compared with the cats that already came to the clinic for regular blood pressure checks after their CKD diagnosis (52%). The overall relatively high incidence of target organ damage provides clinical validity to the diagnosis of hypertension in most cats included in this study. A shorter screening interval can aid in early diagnosis and treatment of hypertension and therefore decrease the development of target organ damage.

Having CKD increases the probability a cat develops systemic hypertension, but their blood pressure does not increase at a significantly greater rate than cats that are healthy and become hypertensive, although this result may have been affected by group size. Cats with CKD included in this study were significantly older than healthy cats that remained normotensive, and even though the difference was not significant for the healthy-DH group, the age of these cats seems comparable to the healthy-NT group. This is a confounding factor of this study, and difficult to control for as CKD prevalence increases with age.[6] There was no correlation between creatinine concentration and SBP when the data from all time points for each cat was used, similar to the result of a cross-sectional study in cats with CKD.[5] A study investigating differences between hypertensive and normotensive cats also reported no significant difference in creatinine concentration between groups.[3]

Human patients with end stage renal disease almost invariably suffer from hypertension, but in human CKD patients there is no correlation between serum creatinine concentration and blood pressure,[25,26] although a

decline in creatinine clearance is mildly correlated with mean blood pressure in healthy subjects.[15] It has been demonstrated that baseline creatinine is an independent risk factor for the development of azotemic CKD in cats.[7] This study shows that creatinine is also an independent risk factor for the development of hypertension and that CKD cats are more likely to be diagnosed with clinically significant hypertension. Historically it has been suggested that hypertension in most cats is secondary to CKD, and several mechanisms, such as activation of the renin-angiotensin-aldosterone system (RAAS) and fluid retention have been described.[11,27,28] A recent study showed that plasma renin activity is suppressed in hypertensive cats, whereas plasma aldosterone concentration is increased, although there was a substantial overlap among groups.[28] However, it could be hypothesized that CKD and hypertension share a pathophysiological basis that is not yet understood. The possibility exists that feline hypertension has a genetic component, like essential hypertension in humans,[29] but to date no studies into the genetics of feline hypertension have been published. This is an area that warrants further investigation.

Conclusion

In conclusion, blood pressure increases with age in all cats. Cats that develop clinically significant hypertension have a higher blood pressure at initial evaluation than their normotensive counterparts and cats with CKD are more likely to develop hypertension. The high prevalence of hypertension in azotemic cats in this study shows the importance of monitoring of SBP in elderly cats and, in particular, in cats with CKD. It could, therefore, be suggested that cats with a higher baseline blood pressure at diagnosis of CKD should be more closely monitored than cats with a lower baseline blood pressure. Creatinine concentration is an independent risk factor for the development of hypertension, and early diagnosis of CKD is essential so that appropriate management can be offered.

Acknowledgments

Footnotes
a　Parks Electronic Doppler Model 811B, Perimed UK, Bury St Edmunds, UK
b　Mistral 3000, Sanyo-Gallenkamp, Leicestershire, UK
c　IDEXX Laboratories, Wetherby, Yorkshire, UK
d　R i386 3.0.1; R Foundation for Statistical Computing, Vienna, Austria and GraphPad Prism 6; GraphPad Software, La Jolla, CA, USA

References

1. Elliott J, Fletcher M, Syme H. Idiopatic feline hypertension: Epidemiological study. *J Vet Intern Med* 2003;17:754.
2. Littman M. Spontaneous systemic hypertension in 24 cats. *J Vet Intern Med* 1994;8:79–86.
3. Syme HM, Barber PJ, Markwell PJ, et al. Prevalence of systolic hypertension in cats with chronic renal failure at initial evaluation. *J Am Vet Med Assoc* 2002;220:1799–1804.
4. Stiles J, Polzin D, Bistner S. The prevalence of retinopathy in cats with systemic hypertension and chronic renal failure or hyperthyroidism. *J Am Anim Hosp Assoc* 1994;30:564–572.
5. Kobayashi DL, Peterson ME, Graves TK, et al. Hypertension in cats with chronic renal failure or hyperthyroidism. *J Vet Intern Med* 1990;4:58–62.
6. Lulich JP, Osborne CA, O'Brien TD, et al. Feline renal failure: Questions, answers, questions. *Compend Contin Educ Pract Vet* 1992;14:127–153.
7. Jepson RE, Brodbelt D, Vallance C, et al. Evaluation of predictors of the development of azotemia in cats. *J Vet Intern Med* 2009;23:806–813. 860 Bijsmans et al
8. Miall WE, Lovell HG. Relation between change of blood pressure and age. *Br Med J* 1967;2:660–664.
9. Rodriguez BL, Labarthe DR, Huang B, et al. Rise of blood pressure with age. New evidence of population differences. *Hypertension* 1994;24:779–785.
10. Sansom J, Rogers K, Wood JL. Blood pressure assessment in healthy cats and cats with hypertensive retinopathy. *Am J Vet Res* 2004;65:245–252.
11. Mishina M, Watanabe T, Fujii K, et al. Non-invasive blood pressure measurement in cats: Clinical significance of hypertension associated with chronic renal failure. *J Vet Med Sci* 1998;60:805–808.
12. Brown S, Atkins C, Bagley R, et al. Guidelines for the indentification, evaluation, and management of systemic hypertension in dogs and cats. *J Vet Intern Med* 2007;21:542–558.
13. Boag AK, Neiger R, Slater L, et al. Changes in the glomerular filtration rate of 27 cats with hyperthyroidism after treatment with radioactive iodine. *Vet Rec* 2007;161:711–715.
14. Elliott J, Barber PJ, Syme HM, et al. Feline hypertension: Clinical findings and response to antihypertensive treatment in 30 cases. *J Small Anim Pract* 2001;42:122–129.
15. Lindeman RD, Tobin JD, Shock NW. Association between blood pressure and the rate of decline in renal function with age. *Kidney Int* 1984;26:861–868.

16. Chetboul V, Lefebvre HP, Pinhas C, et al. Spontaneous feline hypertension: Clinical and echocardiographic abnormalities, and survival rate. *J Vet Intern Med* 2003;17:89–95.

17. Sparkes AH, Caney SMA, King MCA, et al. Inter- and intraindividual variation in doppler ultrasonic indirect blood pressure measurements in healthy cats. *J Vet Intern Med* 1999;13:314–318.

18. Bodey AR, Sansom J. Epidemiological study of blood pressure in domestic cats. *J Small Anim Pract* 1998;39:567–573.

19. Stephens MJ, O'Neill DG, Church DB, et al. Feline hyperthyroidism reported in primary-care veterinary practices in England: Prevalence, associated factors and spatial distribution. *Vet Rec* 2014;175:458.

20. Kim JR, Kiefe CI, Liu K, et al. Heart rate and subsequent blood pressure in young adults: The CARDIA study. *Hypertension* 1999;33:640–646.

21. Paepe D, Verjans G, Duchateau L, et al. Routine health screening: Findings in apparently healthy middle-aged and old cats. *J Feline Med Surg* 2013;15:8–19.

22. Cirillo M, Laurenzi M, Trevisan M, et al. Hematocrit, blood pressure, and hypertension. The Gubbio population study. *Hypertension* 1992;20:319–326.

23. de Simone G, Devereux RB, Chinali M, et al. Risk factors for arterial hypertension in adults with initial optimal blood pressure: The Strong Heart Study. *Hypertension* 2006;47:162–167.

24. Vasan RS, Larson MG, Leip EP, et al. Assessment of frequency of progression to hypertension in non-hypertensive participants in the Framingham Heart Study: A cohort study. *Lancet* 2001;358:1682–1686.

25. Bulpitt CJ, Hodes C, Everitt MG. The relationship between blood pressure and biochemical risk factors in a general population. *Br J Prev Soc Med* 1976;30:158–162.

26. Perneger TV, Nieto FJ, Whelton PK, et al. A prospective study of blood pressure and serum creatinine. *JAMA* 1993;269:488–493.

27. Jensen J, Henik RA, Brownfield M, et al. Plasma renin activity and angiotensin I and aldosterone concentrations in cats with hypertension associated with chronic renal disease. *Am J Vet Res* 1997;58:535–540.

28. Jepson RE, Syme HM, Elliott J. Plasma renin activity and aldosterone concentrations in hypertensive cats with and without azotemia and in response to treatment with amlodipine besylate. *J Vet Intern Med* 2014;28:144–153.

29. Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. *Nat Genet* 2009;41:677–687.

Reprint paper*

Prevalence and prognostic importance of pulmonary hypertension in dogs with myxomatous mitral valve disease

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ABSTRACT

Background: Pulmonary hypertension (PH) is common in dogs with myxomatous mitral valve disease (MMVD) but its effect on clinical outcome has not been investigated.

Hypothesis/objectives: The presence of PH worsens the outcome in dogs with MMVD. To compare survival times of dogs with MMVD and PH to those without PH.

Animals: Two hundred and twelve client-owned dogs.

Methods: Case review study. Medical records of dogs diagnosed with ACVIM stage B2 and C MMVD between January 2010 and December 2011 were retrospectively reviewed. Long-term outcome was determined by telephone interview or from the medical record. End of the observation period was March 2013. PH was identified if tricuspid regurgitation peak velocity was >3 m/s.

Results: Two hundred and twelve were identified. Eighty-three dogs (39%) had PH. PH was more commonly identified in stage C compared to B2 (P < 0.0001). One hundred and five (49.5%) dogs died during the observation period. Median survival time for the entire study population was 567 days (95% CI 512–743). Stage C (P = 0.003), the presence of PH (P = 0.009), left atrial to aortic root ratio (LA/Ao) >1.7 (P = 0.0002), normalized left-ventricular end-diastolic diameter (LVEDn) >1.73 (P = 0.048), and tricuspid regurgitation pressure gradient (TRPG) >55 mmHg (P = 0.009) were associated with worse outcomes in the univariate analyses. The presence of TRPG >55 mmHg (HR 1.8 95% CI 1-2.9; P = 0.05) and LA/Ao > 1.7 (HR 2 95% CI 1.2-3.4; P = 0.01) remained significant predictors of worse outcome in the multivariate analysis.

Conclusions and Clinical Importance: In dogs with MMVD, moderate to severe PH worsens outcome.

Keywords: Canine, Doppler echocardiography, Echocardiography, Heart failure, Heart valve

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Abbreviations

CHF	congestive heart failure		LVEDDn	normalized left-ventricular end-diastolic diameter
CI	confidence interval		LVESDn	normalized left-ventricular end-systolic diameter
E	max peak velocity of E wave of transmitral flow		MMVD	myxomatous mitral valve disease
HR	hazard ratio		TR	velocity of tricuspid regurgitation
Edt	E-wave deceleration time of transmitral flow		PH	pulmonary hypertension
HF	heart failure		RVenl	right-ventricular enlargement
LA/Ao	left atrial to aortic root ratio		TRPG	peak tricuspid regurgitation pressure gradient

Introduction

Pulmonary hypertension (PH) is a common complication in dogs affected by myxomatous mitral valve disease (MMVD).[1] PH caused by left-sided heart failure (HF) is initially the consequence of passive back transmission of increased left-ventricular filling pressure to the pulmonary capillaries. This stage is generally considered reversible. However, if pulmonary venous pressure remains increased or continues to increase, pulmonary artery vasoconstriction and then pulmonary artery and vein remodelling might occur, at which time PH can become irreversible.[2]

Right-heart catheterization is considered the 'gold standard' for diagnosis of PH.[3] In veterinary medicine, right-heart catheterization is rarely performed in routine practice, and echocardiography is the standard non-invasive technique for the diagnosis of PH.[4] PH is associated with a worse prognosis in human patients with left-sided HF, including those with MMVD.[5,6] In veterinary medicine, there are currently no data regarding the prognostic importance of PH in dogs with MMVD. Accordingly, we hypothesized that dogs with stage B2 and C MMVD and echocardiographically identified PH have shorter survival time than dogs with MMVD that do not have PH. The objective of this study was to compare survival times of dogs with PH to those without PH in a population of dogs with ACVIM stages B2 or C MMVD.[7]

Materials and Methods

The medical records of dogs examined between January 2010 and December 2011 at seven referral cardiology centres in United States, Italy and Sweden, were reviewed. Search criteria included dogs with ACVIM stage B2 or C MMVD with or without an echocardiographic diagnosis of PH.

Inclusion Criteria

Dogs with ACVIM Stage B2 or Stage C MMVD that had been subject to physical examination, thoracic radiography, and echocardiography were considered for inclusion in the study population. Echocardiographic inclusion criteria were: 2-D detection of mitral valve prolapse, any degree of mitral valve leaflet thickening or both; colour Doppler identification of any degree of mitral valve regurgitation; and M-mode left-ventricular fractional shortening > 20%; left aortic root ratio (LA/Ao) > 1.5,[8] normalized end-diastolic left-ventricular diameter > 1.73, or both.[9] The presence of tricuspid valve regurgitation (TR) was an absolute inclusion criterion; all included dogs had colour Doppler evidence of TR. Dogs were classified as ACVIM stage B2 MMVD if they did not have present or past clinical signs of congestive heart failure (CHF) or radiographic evidence of pulmonary oedema or pulmonary venous congestion. Dogs were classified as ACVIM stage C MMVD if they had presented with past or current clinical signs of CHF in conjunction with past or current evidence of pulmonary oedema, and pulmonary venous congestion on thoracic radiographs.

Exclusion Criteria

Dogs were excluded if they had congenital heart disease or other acquired cardiovascular disorders such as bacterial endocarditis or dilated cardiomyopathy. Dogs with MMVD without cardiac remodelling (stage B1) and those with MMVD and refractory CHF (stage D) were also excluded. Dogs with stage D have often right-sided HF and decreased right-ventricular function. In these circumstances, the Doppler-derived estimated pulmonary artery pressure does not adequately reflect pulmonary vascular resistance and might underestimate the severity of PH.[6] Dogs were also excluded if they had a history of clinically symptomatic systemic diseases or identifiable causes of PH other than MMVD such as clinically evidence of respiratory disease.

Clinically evidence of respiratory disease was defined as the presence of moderate to marked bronchial, interstitial or alveolar pulmonary pattern in dogs that had diffuse pulmonary crackles on physical examination. Dogs with tracheal collapse were also excluded, as were dogs with a history of a positive heartworm test.

Baseline Data

Data obtained from the case records were: breed, sex, age, body weight, and treatment at the time of examination. Echocardiographic data retrieved were: normalized left-ventricular end-diastolic (LVEDDn) and end-systolic diameter (LVESDn),[9] LA/Ao, peak E-wave velocity (E max) and E-wave deceleration time (Edt) of the transmitral flow, peak velocity of tricuspid regurgitation (peak TR) from whatever echocardiographic view provided the highest velocity and the presence of right-ventricular enlargement (RVenl). Right-ventricular enlargement was subjectively assessed based on 2-D echocardiographic examination. The clinical dataset was reviewed by a single investigator (MB).

Echocardiographic Measurements

The LA/Ao was obtained from the 2-D short-axis view.[8,10] LVDDn and LVESDn were calculated according to Cornell's method of allometric scaling: $LVEDDn = LVEDD/BW^{0.294}$ and $LVESDn = LVESD/BW^{0.315}$.[9] Right-ventricular enlargement was subjectively assessed and classified as yes/no. Assessment of TR by colour Doppler was obtained with the aliasing velocity set between 60 and 90 cm/s. When Doppler spectrograms of TR were not recorded or were not measurable, dogs were considered not affected by PH if they had trivial TR and there was no evidence of RVenl. Peak velocity of TR was obtained under colour Doppler guidance. Systolic pulmonary pressure was estimated calculating the peak tricuspid regurgitation gradient (TRPG) using the simplified Bernouilli equation: $TRPG = 4 \times TR$.[2] PH was diagnosed if the peak TR velocity was >3 m/s corresponding to a TRPG <36 mmHg.

Clinical Progress and Survival

Investigators, trained senior veterinary students or veterinary technician conducted telephone interviews with dog owners or reviewed medical records to determine the clinical outcome of each dog. Clinical progression of dogs for which it was not possible to contact the owner was assessed by review of medical records. Survival time was counted from the day of diagnosis of stage B2 or C MMVD to either the day of death or closing time of the study (March 31, 2013). End-point of the study was death (all causes).

Statistical Analysis

Baseline descriptive statistics were presented as mean and standard deviation for normally distributed continuous variables, whereas non-normally distributed variables were presented as median and range. Categorical variables were analysed with Chi-Square analysis. Between-group analyses of baseline variables were performed using analysis of variance or test for median as appropriate for the error residuals distribution. Effects on survival of the following variables at baseline were evaluated: ACVIM stage, presence of PH, LA/Ao > 1.7, LVEDDn > 1.73, LVESDn > 1.4, TRPG > 55 mmHg. The 55 mmHg TRPG was chosen based on visual inspection of penalized spline hazard plots of pressure gradient modelling mortality on a logarithmic scale relative to the absolute value on a linear scale for TRPG suggesting a linear increase in hazard for values >50 mmHg (Fig 1). Time-to-event analyses were carried out in univariate analysis by way of Kaplan-Meier product limit estimates. Dogs that were lost to follow-up before closing time of the study were censored after last visit. Cox semi-parametric regression models were used to generate multivariate models. When developing multivariate models continuous variables were categorized by visual inspection of log hazards plotted on a continuum by way of penalized spline with four degrees of freedom and based on clinical rationale and previous literature. Model relative goodness of fits was analysed by Akaike information criterion and compared using a Chi-Square one degree of freedom test. Tests for proportionality were carried out by visual inspection of Schoenfeld residuals, negative log estimated SDF, and formal hypothesis testing of covariate by log (time) interactions followed by Wald Chi-Square statistics and deemed proportional. Additional sensitivity analyses were carried out using parametric accelerated time failure models affirmatively validating the Cox proportionality assumptions. All analyses were deemed significant at $P \leq 0.05$ and carried out using a commercial software.[a]
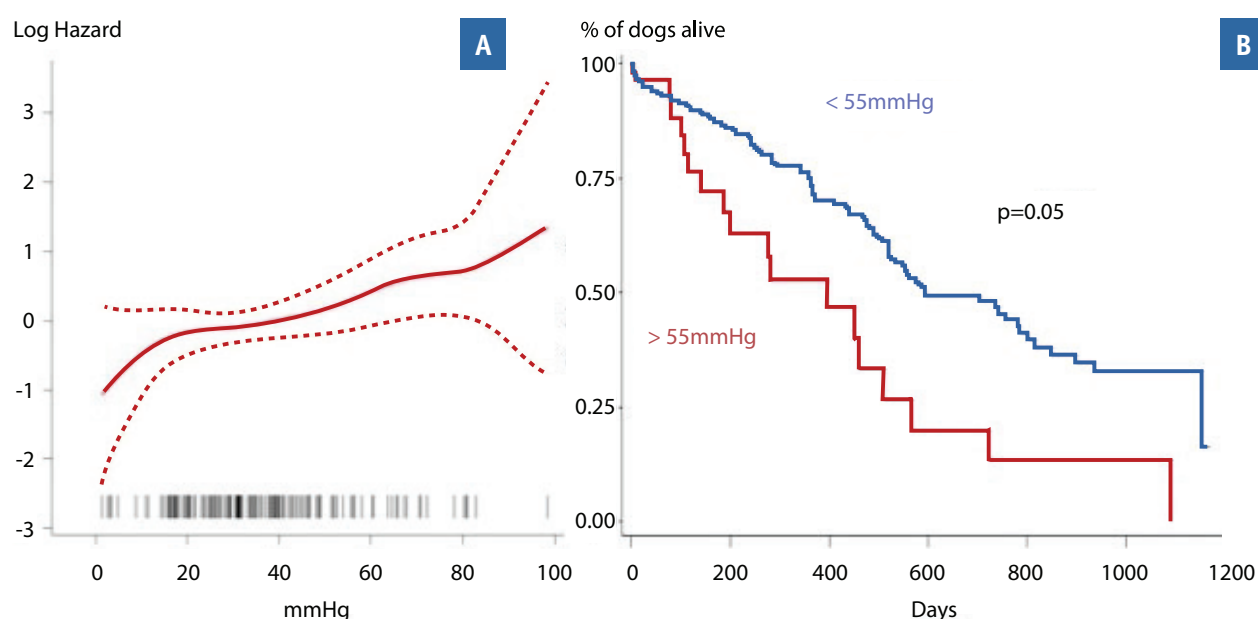
Figure 1: (A) Penalized spline (df = 4) hazard plots of pressure gradient modelling death on a logarithmic scale relative to the absolute value on a linear scale for trans–tricuspid pressure gradient (TRPG). Visual inspection suggest a linear increase in the hazard for values of TRPG > 50 mmHg. Yellow graph lines represent 95% Confidence interval (95% CI). (B) Graph depicting survival times as Kaplan–Meier curves for TRPG greater and less than 55 mmHg.

Results

Baseline Characteristics

Baseline characteristics for the general population and for dogs with and without PH are summarized in Table 1.

Two hundred and twelve dogs, 121 (57%) males (41 neutered) and 91 (43%) females (67 spayed) from 41 breeds were included in the study. In decreasing magnitude of representation, breeds included: mixed (n = 48; 23%), Cavalier King Charles spaniel (n = 30; 14%), Dachshunds

Table 1 Descriptive statistics for all 212 dogs, dogs without pulmonary hypertension (PH), and dogs with PH

	All (n = 212)	No PH (129)	PH (n = 83)	P-Value
Age (years) (n = 212)	10.6 ± 2.6	10.6 ± 2.6	10.7 ± 2.7	0.94
Sex (F/M) (n = 212)	91/121 (43%/57%)	55/74 (43%/57%)	36/57 (43%/57%)	0.91
Weight (kg) (n = 212)	8.6 (1.2–80.7)	9.1 (1.2–80.7)	7.7 (1.6–67)	0.19
ACVIM stage (n = 212)	100 B2 (47%) 112 C (53%)	76 B2 (59%) 53 C (41%)	24 B2 (29%) 59 C (71%)	0.0022 0.0023
LA/Ao (n = 211)	2 (1.3–3.9)	1.9 (1.3–3.2)	2.3 (1.4–3.9)	< 0.0001
LVEDDn (n = 211)	2.02 (0.8–2.9)	1.9 (0.9–2.9)	2.1 (0.8–2.9)	0.006
LVESDn (n = 211)	1.05 (0.2–1.9)	1.04 (0.4–1.9)	1.04 (0.2–1.8)	0.877
E peak (m/s) (n = 117)	1.28 ± 0.40	1.3 ± 0.3	1.42 ± 0.37	0.08
TRPG (mmHg) (n = 191)	33.2 (1.4–98.4)	24.1 (1.4–35.5)	46.2 (36.0–98.4)	< 0.0001
RV enlargement (yes/no) (n = 123)	15 (12%)	7(47%)	8 (53%)	0.067

P-value is referring to differences between dogs with and without PH. No PH, no pulmonary hypertension, ACVIM stage, class of heart failure according to ACVIM classification; LA/Ao, left-atrial to aortic root ratio; LVEDDn, normalized left-ventricle end-diastolic diameter indexed; LVESDn, normalized left-ventricle end-systolic diameter indexed; E peak, peak velocity of E wave of transmitral flow; TRPG, tricuspid regurgitation pressure gradient; RV, right ventricle.

(n = 16; 7%) and Miniature Poodle (n = 12; 6%). One hundred dogs were in stage B2 (47%) and 112 (53%) were in stage C of MMVD. PH was diagnosed in 83 (39%) dogs. Age, weight, LVESDn, E max and presence/absence of RVenl were not different between dogs with and without PH. PH was significantly more common in dogs with stage C MMVD (24; 29% stage B2, 59; 71% stage C; P < 0.001). Dogs with PH had significantly larger left atria, LVEDDn and TRPG compared to dogs without PH (Table 1). At baseline, 131 (62%) dogs were receiving medical treatment for heart disease (41 stage B2 and 90 stage C). Twenty seven dogs received furosemide in combination with an ACE-I, 48 dogs furosemide in combination with an ACE-I and pimobendan, 5 dogs furosemide in combination with an ACE-I, pimobendan and spironolactone. Of dogs that received only a single drug, one received furosemide, 18 dogs received an ACE-I, 2 dogs, pimobendan and 3 dogs spironolactone. All dogs received at least an ACE-I, pimobendan and furosemide after diagnosed with ACVIM stage C.

Survival Analysis and Progression

During the observation period, 105 dogs (49.5%) died or were euthanized because of refractory CHF. Of these dogs 26 were ACVIM stage B2 and 79 ACVIM stage C MMVD. The median survival time was 567 days (95% CI 512-743). Median survival times for stage B2 and C dogs were 784 days (95% lower CI 576, upper CI non-estimable) and 491 days (95% CI 440-561), respectively. The median survival time for dogs without PH was 758 days (95% CI 527-848) and for those with PH, 456 days (95% CI 360-567). Of the six variables used as predictors in the univariate analysis, ACVIM stage C (HR 1.8, 95% CI 1.2-2.7; P = 0.003), the presence of PH (HR 1.6 95% CI 1.1-2.4; P = 0.009), LA/Ao > 1.7 (HR 2.4, 95% CI 1.5-4; P = 0.0002), LVEDDn > 1.73 (HR 1.7, 95% CI 1-1.7; P = 0.048), and TRPG > 55 mmHg (HR 2.3 95% CI 1.4-3.8; P = 0.002) were associated with worse outcomes (Figs 1, 2). The presence of TRPG > 55 mmHg (HR 1.8 95% CI 1-2.9; P = 0.05) and LA/Ao > 1.7 (HR 2 95% CI 1.2-3.4; P = 0.01) remained significant predictors of poor outcome in the multivariate analysis.

Discussion

Results of this study demonstrate that echocardiographic evidence of PH is common in dogs with ACVIM stage B2 and C MMVD and that it is associated with a poorer prognosis. Prevalence of detectable and quantifiable PH in these population of dogs was 39%. Previous studies have reported a prevalence of PH in dogs with MMVD

between 14 and 53%.[11, 12, 13, 14] One possible reason for the difference observed between studies is the use of a different TR velocity cut off to establish the presence of PH. In this study PH was diagnosed if TR velocity was more than 3 m/s. Other studies have used a lower cut off.[12,13] Prevalence of the disease is also related to the population studied. One large study, reported a lower prevalence of PH in a population of 617 dogs with different stages of MMVD.[12] However, in that study, more than half of the included dogs had a mild form of MMVD not associated with cardiac remodelling. Two studies have reported that the prevalence of PH was associated with severity of MMVD.[12,15] Similarly, in this study, dogs with stage C MMVD had a significantly greater prevalence of PH compared to dogs in stage B2. This is not surprising as PH with left-sided heart disease is initially the consequence of passive back transmission of increased left-ventricular filling pressure to the pulmonary capillaries.[2] Accordingly, humans and animals with more severe mitral regurgitation, and therefore higher left-atrial pressure, have an increased risk of developing PH. In our study about one third of dogs with PH were in stage B2; this finding suggests that a certain number of dogs considered pre-clinical for left-sided HF have high left-atrial pressure. Dogs with stage B2 MMVD represents a population that is heterogeneous with respect to cardiac remodelling; in some dogs, cardiac enlargement is mild and in others, severe, despite the fact, that clinical signs are, by definition, absent. Therefore, it is possible that dogs with more severe remodelling have higher left-atrial pressure and are actually in left-sided HF but clinical signs were missed by the owner. More objective measures of clinical signs might help identify this subset of dogs. Recently two studies have suggested that resting respiratory rate represent a useful objective clinical variable to identify dogs with left-sided HF.[14,16]

Univariate analysis indicated that stage of the disease, left-atrial enlargement, left-ventricular enlargement, presence of PH and a TRPG > 55 mmHg were predictors of survival. However, only left-atrial enlargement and a TRPG > 55 mmHg were predictors of death in the multivariate analysis. In dogs with MMVD, left-atrial size is related to severity of MR and is an independent predictor of progression and death.[17,18,19] Degree of left-atrial enlargement has also been associated with severity of PH in dogs with MMVD.[12,15] The fact that PH was not an independent predictor of death in multivariate analysis is likely because of the fact it is correlated with left-atrial enlargement. However, TRPG > 55 mmHg remained an

independent negative predictor of survival. If pulmonary venous pressure remains increased or continues to increase in humans and animals with left-sided HF, local and systemic release of angiotensin II, tumour necrosis factor, endothelin-1 together with impaired nitric-oxide mediated pulmonary vasodilation lead to pulmonary artery and vein remodelling and the process becomes irreversible.[2] In these humans and animals, pulmonary vascular resistance is increased and they develop PH that is out of proportion to left-atrial pressure or, 'reactive PH'.[3] A systolic pulmonary arterial pressure >50 mmHg has been reported to be associated with poor outcome in human beings with MMVD.[5] In dogs with MMVD, a TRPG at or above 48 mmHg suggests the presence of irreversible PH.[15] Another study in humans with cardiomyopathies demonstrated that each 5-mmHg increase in baseline of mean pulmonary arterial pressure, increased hazard of death by 25%.[20] Similarly, in this study, visual inspection of spline curve for TRPG shows that there is a linear increase for the risk of death with TRPG more than 50 mmHg (Fig 2). Concomitant chronic respiratory diseases, such as tracheal collapse, chronic bronchitis or interstitial lung disease, also commonly affect small breed dogs with MMVD.[21] These diseases might contribute to the development of PH in dogs with MMVD. Therefore, it is possible that PH in some of the dogs included in this study could be induced by concomitant respiratory disease. However, none of the included dogs had a history, clinical signs, and radiographic evidence of severe respiratory disease and this study did not include dogs of breeds known to be predisposed to interstitial lung disease. Moreover, our results show that dogs with more advanced MMVD were more likely to have PH. These data indirectly support the contention that MMVD is most likely the cause of PH in this sample population.

This study has some limitations. First, diagnosis of PH was only based on peak velocity of TR. Right-heart catheterization is considered the 'gold standard' for the definitive diagnosis of PH.[3] However, in veterinary medicine right-ventricular catheterization is rarely performed in routine practice and PH hypertension is generally diagnosed based on the presence of clinical and echocardiographic findings. Moreover, peak velocity of TR is affected by right-ventricular contractility.[3,4] In this study, we reported if the right-ventricle was subjectively enlarged but right-ventricular function was not assessed. Second, TRPG might be underestimated in some dogs because of technical difficulties in obtaining an ideal alignment with eccentric tricuspid regurgitant
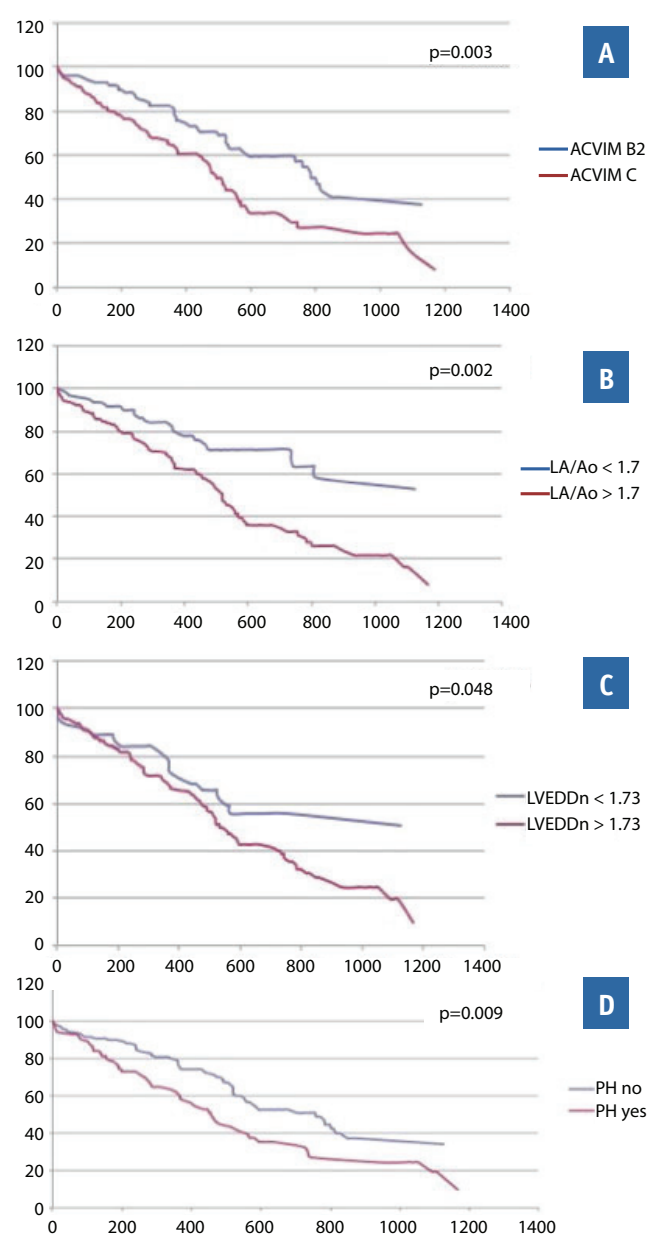

Figure 2: Graphs depicting survival times as Kaplan–Meier curves for variable affecting survival in dogs with myxomatous mitral valve degeneration. (A) myxomatous mitral valve disease (MMVD) dogs in ACVIM stage B2 and C. (B) Dogs with left-atrial to aortic root ratio (LA/Ao) > 1.7. (C) Dogs with normalized left-ventricular end-diastolic dimension (LVEDDn) > 1.73. (D) Dogs with and without pulmonary hypertension (PH).

jets. In this study assessment of TR was obtained from the echocardiographic view providing the highest peak velocity and best alignment. Third, TR might not be present in all dogs with PH. Fourth, this study has a retrospective design. Retrospective studies increase the risk of uncontrolled systematic errors. Furthermore, because of the retrospective design, all variables were not available at the baseline. Finally, because of the many combinations of drugs administered, and the retrospective nature of

the study, it was not possible to analyse the effects of treatment in the studied population. However, all stage C dogs were treated with standard treatment including an ACE-I, pimobendan and furosemide after their inclusion in the study.

Clinical Relevance

This study demonstrated that PH is a commonly associated with stage B and C MMVD in dogs and that the presence of PH is associated with an increased risk of death. Furthermore, the finding that about one third of dogs with PH in this study were classified as ACVIM stage B2 suggests that this stage of the disease includes a heterogeneous group of dogs.

Acknowledgments

Conflict of Interest Declaration:
Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration:
Authors declare no off-label use of antimicrobials.

Footnote
[a] SAS 9.3 (Cary, NC 2013)

References

1. Kellihan HB, Stepien RL. Pulmonary hypertension in dogs: Diagnosis and therapy. *Vet Clin North Am Small Anim Pract* 2010;40:623–641.
2. Guazzi M, Arena R. Pulmonary Hypertension with left sided heart disease. *Nat Rev Cardiol* 2010;7:648–659.
3. Galie N, Hoeper MM, Humbert M, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Respir J* 2009;34:1219–1263.
4. Kellihan HB, Stepien RL. Pulmonary hypertension in canine degenerative mitral valve disease. *J Vet Cardiol* 2012;14:149–164.
5. Barbieri A, Bursi F, Grigioni F, et al. Prognostic and therapeutic implications of pulmonary hypertension complicating degenerative mitral regurgitation due to flail leaflet: A multicenter long-term international study. *Eur Heart J* 2011;32:751–759.
6. Ghio S, Gavazzi A, Campana C, et al. Independent and additive prognostic value of right ventricular systolic function and pulmonary artery pressure in patients with chronic heart failure. *J Am Coll Cardiol* 2001;37:183–188.
7. Atkins C, Bonagura J, Ettinger S, et al. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. *J Vet Intern Med* 2009;23:1142–1150.
8. Hansson K, Haggstrom J, Kvart C, et al. Left atrial to aortic root indices using two-dimensional and M-mode echocardiography in Cavalier King Charles Spaniels with and without left atrial enlargement. *Vet Radiol Ultrasound* 2002;43:568–575.
9. Cornell CC, Kittleson MD, Della Torre P, et al. Allometric scaling of M-mode cardiac measurements in normal adult dogs. *J Vet Intern Med* 2004;18:311–321.
10. Rinshiw M, Hollis NE. Evaluation of four 2-D dimensional echocardiographic methods of assessing left atrial size in dogs. *J Vet Intern Med* 2000;14:429–435.
11. Borgarelli M, Zini E, D'Agnolo G, et al. Comparison of primary mitral valve disease in German Shepherd dogs and in small breeds. *J Vet Cardiol* 2004;6:27–34.
12. Serres FJ, Chetbul V, Tissier R, et al. Doppler echocardiography-derived evidence of pulmonary arterial hypertension in dogs with degenerative mitral valve disease: 86 cases (2001–2005). *J Am Vet Med Assoc* 2006;229:1772–1778.
13. Guglielmini C, Civitella C, Diana A, et al. Serum cardiac Tn I concentration in dogs with with precapillary and post capilllary pulmonary hypertension. *J Vet Intern Med* 2010;24:145–152.
14. Schober KE, Hart TM, Stern JA, et al. Detection of congestive heart failure in dogs by Doppler echocardiography. *J Vet Intern Med* 2010;24:1358–1368.
15. Chiavegato D, Borgarelli M, D'Agnolo G, et al. Pulmonary hypertension in dogs with mitral regurgitation attributable to myxomatous valve disease. *Vet Radiol Ultrasound* 2009;50:253–258.
16. Ohad DG, Rishniw M, Ljungvall I, et al. Sleeping and resting respiratory rates in dogs with subclinical heart disease. *J Am Vet Med Assoc* 2013;243:839–843.
17. Borgarelli M, Savarino P, Crosara S, et al. Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. *J Vet Intern Med* 2008;22:120–128.
18. Haggstrom J, Boswood A, O'Grady M, et al. Effect of pimobendan or benazepril hydrochloride on survival times in dogs with congestive heart failure caused by naturally occurring myxomatous mitral valve disease: The QUEST study. *J Vet Intern Med* 2008;22:1124–1135.
19. Borgarelli M, Crosara S, Lamb K, et al. Survival characteristics and prognostic variables of dogs with preclinical chronic degenerative mitral valve disease attributable to myxomatous degeneration. *J Vet Intern Med* 2012;26:69–75.
20. Cappola TP, Felker GM, Kao WH, et al. Pulmonary hypertension and risk of death in cardiomyopathy: Patients with myocarditis are at higher risk. *Circulation 2*002;105:1663–1668.

21. Ferasin L, Crews L, Biller DS, et al. Risk factors for coughing in dogs with naturally acquired myxomatous mitral valve disease. *J Vet Intern Med* 2013;27:286–292.

Reprint paper*

Haemostatic variables, plasma lactate concentration, and inflammatory biomarkers in dogs with gastric dilatation-volvulus

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SUMMARY

Objective: Prospective characterization of haemostatic variables, plasma lactate concentration, and inflammatory biomarkers in dogs with gastric dilatation-volvulus (GDV). Material and methods: Coagulation variables (platelets, prothrombin time, activated partial thromboplastin time, fibrinogen, antithrombin [AT], protein C [PC], protein S [PS], D-dimers), plasma lactate concentration and inflammatory biomarkers (C-reactive protein, white blood cell count, lymphocyte and neutrophil numbers) were assessed in 20 dogs with GDV presented between 2011 and 2012. Blood was taken pre-operatively and at days 1 and 3 postoperatively. The prognostic value of these variables before and after surgery was evaluated as well as the behaviour of variables during the study.

Results: Overall, 7/20 (35%) dogs did not survive; two dogs (29%) were euthanized during surgery due to severe gastric necrosis and 5 (71%) dogs after surgery due to sepsis and disseminated intravascular coagulopathy. Prior to surgery, median plasma lactate concentration was significantly (p = 0.01) lower in survivors (6.2 mmol/l, range 1.9–9.7 mmol/l) when compared to non-survivors (11.8 mmol/l, range 7.5–16.2 mmol/l). In dogs dying after surgery, significantly higher plasma lactate concentration, coagulation times and D-dimer concentration were present as well as lower fibrinogen concentration and activity of PC and AT compared to survivors. At discharge, activity of AT, PC and PS were markedly below the reference interval in 6/13 (46%), 11/13 (85%), and 8/13 (62%) dogs, respectively. Clinical relevance: Only lactate plasma concentration was of preoperative prognostic value. After surgery, severe abnormalities of coagulation variables, especially the endogenous anticoagulants were present in most of the dogs. The severity of the abnormalities was associated with survival.

Keywords: Dog, haemostasis, protein C, protein S, antithrombin

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Introduction

Gastric dilatation-volvulus (GDV) seems to activate coagulation, which subsequently might lead to a decrease and depletion of endogenous anticoagulants such as protein C (PC) and antithrombin (AT)[30], and finally to an overt disseminated intravascular coagulopathy (DIC) [2]. The latter has been proven to significantly enhance mortality in dogs with GDV[6,9]. Early diagnosis and treatment of DIC including the treatment of the underlying cause significantly improves outcome[38]. Despite this knowledge, few studies focus on alterations of haemostatic profiles including platelet count (PLT), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (Fib), AT and D-dimer concentrations in dogs with GDV[1,33]. To our knowledge, an extended coagulation profile including several endogenous anticoagulants such as AT, PC, and protein S (PS) has not been evaluated before. Moreover, characterization of coagulation response by serial measurements during the course of disease has been rarely performed in dogs. It is well known that there is a close bidirectional interaction between inflammation and coagulation response[21]. Previous experimental[17] and clinical[5,8,14,15] investigations evaluating the influence of systemic inflammatory processes on coagulation response in healthy dogs reported a marked decrease in natural anticoagulants PC and AT. In dogs with sepsis, survivors seem to have higher anticoagulant activities[15], which is comparable to humans where deficiencies of endogenous anticoagulants PC and AT are associated with higher mortality[19,31]. To our knowledge, the interaction between inflammatory response and coagulation as well as the prognostic value of an extended coagulation profile and markers of inflammation such as white blood cell count (WBC) and C-reactive protein (CRP) has not been evaluated in dogs with GDV.

It was the purpose of this study to evaluate the prognostic value of variables reflecting all phases of coagulation, the plasma lactate concentration reflecting tissue hypoxia and inflammatory biomarkers in dogs with GDV. The overall prognostic value of markers for survival before surgery was assessed ('overall survival', i.e. included are all dogs with GDV and a comparison between dogs surviving surgery until discharge and dogs dying during or after surgery is performed) as well as the prognostic value of markers for survival after surgery ('survival until discharge', i.e. just the dogs that survived surgery are included and a comparison between dogs surviving until discharge and dogs dying

after surgery is performed). In addition, the behaviour of these variables prior to surgery until discharge was characterized.

Our hypothesis was that dogs with GDV would show severe abnormalities of the extended coagulation profile consistent with a consumption disorder and that alterations in the coagulation profile would be more severe in non-survivors than in survivors.

Material and methods

Study design

This prospective study was approved by the Ethics Committee for Animal Welfare, administrative office for veterinary affairs and consumer protection, Giessen, Germany (Number: 64–2011). Dogs with GDV treated at the Clinic for Small Animals, Department of Veterinary Surgery, Faculty of Veterinary Medicine, Justus-Liebig-University, Giessen between 2011 and 2012 were included. Inclusion criterion was diagnosis and treatment of GDV as well as sample taking for blood analysis. Dogs, which had been treated by the referring veterinarian or received intravenous fluids before sample taking were excluded from the study. The diagnosis of GDV was based on physical examination and confirmed by lateral abdominal radiographs.

Routine preoperative treatment consisted of stabilization of the dog with intravenous fluid therapy (60–80 ml/kg isotonic saline solution, Sterofundin®, Braun, Melsungen, Germany) and needle gastric decompression. Premedication for anaesthesia included a combination of levomethadone (0.2–0.5 mg/kg IV) and diazepam (0.5–1.0 mg/kg IV). Propofol (2–4 mg/kg IV) was administered for induction, intubation was performed, and anaesthesia was maintained with isoflurane (1.5 vol.%) in pure oxygen (600–800 ml/min). Fluid therapy was continued intraoperatively at a rate of 10–20 ml/kg/h. Appropriate fluid rate and fluid composition were adjusted as needed based on each individual case, i.e. heart rate and indirect blood pressure both measured continuously during surgery (SurgiVet® Advisor® Vital Signs Monitor V9212AR, Smiths Medical PM, Inc, Norwell USA). Hypotensive dogs (systolic blood pressure < 80 mmHg or mean arterial pressure < 60mmHg) were treated additionally with hypertonic saline solution (1–2 ml/kg/h) (HAES®, Braun, Melsungen, Germany). Surgical treatment always

included an exploratory laparotomy, gastric decompression by orogastric tube, repositioning of the stomach, and incorporating gastropexy. Duration of anaesthesia as well as postoperative diagnosis of DIC was documented. Dogs were considered to have DIC if they had at least three of the following five signs: thrombocytopenia, prolonged PT, prolonged aPTT, low plasma fibrinogen concentration, and high D-dimer concentration[5,34]. After surgery, central venous pressure was monitored by placement of a central venous catheter to quantify fluid balance. During hospitalization, vital signs and electrocardiography were assessed twice a day. Dogs were medicated with perioperative antibiotics (amoxicillin-clavulanic acid, 20 mg/kg IV BID) and additional pain management (metamizole sodium; 30–50 mg/kg IV TID). Blood samples were taken preoperatively (T0), at day 1 (T1) and day 3 after surgery (T2) to identify possible preoperative and postoperative prognostic markers as well as to characterize the behaviour of markers in overall survivors.

Coagulation variables reflecting procoagulant activity were assessed by measuring PLT, PT, aPTT and Fib. The endogenous anticoagulant response was assessed by measuring AT, PC and PS. Fibrinolysis was assessed by measurement of D-dimer concentration. Additionally, biomarkers of inflammation were assessed including CRP and WBC count, neutrophil and lymphocyte numbers.

Study group

In total, 20 dogs (12 male, 8 female, 3 neutered males and 2 spayed females) were enrolled in the study. The age ranged between 8 months and 12 years (median 8 years) and the body was between 22 and 45 kg (median 30 kg). The most represented breeds were the Bernese Mountain Dog (4/20, 20%) and German Shepherd Dog (3/20, 15%).

Blood sample collection
At T0, blood was collected from the cephalic vein via a 20-gauge venous catheter (Vasofix® Braunüle, Braun, Melsungen, Germany) and was allowed to drop freely into the tubes. The following blood collections were performed during morning rounds through a central venous catheter (Cervafix® Certo® with Splittocan®, Braun, Melsungen, Germany) in the jugular vein. The catheter was flushed with 0.9% saline and the first 5 ml were discarded. Blood was then aspirated with a 5 ml plain polyethylene syringe (BD Discardit II, LUER TIP, Franklin Lakes, USA)

and was placed in the respective tubes. The first sample was taken for blood gas analysis to assess plasma lactate concentration with tubes containing lithium-heparin (Li-Heparin LH, 1.3, Sarstedt, Nümbrecht, Germany). For haematological analysis samples were placed into a 1.2 ml tube containing potassium ethylendiamine tetra-acetate (K2-EDTA, Sarstedt, Nümbrecht, Germany). The following blood was taken into a 1.2 ml citrated tube containing 3.18% sodium citrate (Sarstedt, Nümbrecht, Germany) such that a ratio of 9 : 1 (vol/vol) was obtained. It has been demonstrated previously that the sampling technique has no impact on the results of the coagulation variables assessed here[4].

Tested parameters
Coagulation variables were analysed with the automated coagulation analyser STA Compact (STA Compact™, Roche Diagnostics GmbH, Mannheim, Germany). Within 1 hour after sampling, citrated whole blood for coagulation analysis was centrifuged for 10 minutes at 850 g. Citrated plasma was then stored at −20 °C until the following working day. Then, it was transferred to another freezer and stored at −80 °C until analysis. Analysis was performed batch-wise 12 months after sampling. Sample stability was proven for this time range previously[3]. Before analysis, samples were thawed at 37 °C in a water bath to completely dissolve the cryoprecipitate as recommended previously[10] and centrifuged at 850 g for 10 minutes. Test methods and internal quality control were performed as reported elsewhere[3,5].

Analysis of plasma lactate concentration was performed with the blood gas analyser cobas b 221 (blood Gas System, Roche Diagnostics GmbH, Basel, Switzerland) directly after blood collection.

Analysis of inflammatory biomarkers was performed with the laser-based haematology system ADVIA 2120 (Siemens Healthcare, Eschborn, Germany) 1 hour after blood collection. Specimens taken during emergency duty were assessed with the laser based in-house haematology analyser ProCyte Dx (IDEXX, Laboratories, Westbrook, ME, USA). An excellent correlation between the PLT count obtained with the ADVIA 2120 analyser and the ProCyte Dx analyser has been demonstrated before[24].

CRP was measured with the analyser Horiba Pentra 400 (ABX Diagnostics, Montpellier, France) with a human test kit (Randox, CRP, Cat. No. CP 9742, Crumlin, United

Kingdom) and a dog calibrator (Life Diagnostics, Inc., Lot nr. C-F1411B) validated previously for its use in dogs[27]. Within 1 hour after sampling, serum was centrifuged for 1 minute at 18,000 g. Storage of the blood serum was similar to that for testing coagulation variables.

Statistical analysis

Statistical analysis was performed with the statistical software packages Graph Pad Prism (Graph Pad Software, San Diego, USA). The results were depicted in comparison with laboratory reference intervals for the respective variables. For all evaluated variables, reference intervals were published previously[3]. A Kolmogorov-Smirnov test was performed to verify the assumption of normality. For all blood variables, a non-normal distribution was present. For serial evaluation of blood variables, a Friedman test and Dunn's multiple comparison post test was used. For the evaluation of overall survival and survival until discharge, a Wilcoxon-Mann-Whitney U-test was used. For all statistical tests, level of significance was set at α ≤ 0.05.

Results

Mean duration of anaesthesia was 80 minutes (range 40–130 minutes). Of 20 dogs, 35% (7/20) did not survive. Two dogs were euthanized during surgery (severe gastric necrosis) and five dogs were euthanized after surgery (sepsis, DIC). Postoperatively, disseminated intravascular coagulation (DIC) was diagnosed in 35% (7/20) of the dogs of which 57% (4/7) did not survive. Median hospitalization time of dogs with DIC was 4 days (range 2–8 days). In 10/20 dogs, fluid therapy was adjusted with hypertonic saline solution (HAES®, Braun, Melsungen, Germany) during surgery until the day after surgery (mean 21 hours, range 18–30 hours).

Prior to surgery, the median plasma lactate concentration was significantly higher in overall non-survivors in contrast to overall survivors, however, the other evaluated variables were not significantly different between the groups (see Table 1).

Table 1 Lactate, C-reactive protein (CRP), haematological variables and coagulation variables in overall survivors (n = 13) and non-survivors (n = 7) prior to surgery. WBC = white blood cells, PLT = platelets; PT = prothrombin time; aPTT = activated partial thromboplastin time. Significant p-values are marked in bold.

| Variable (unit)	Reference interval	Median (range)		p value
		Survivors	Non-survivors	
PLT (10⁹/l)	150–500	192 (93–315)	141 (109–250)	0.32
PT (sec)	5.7–8.0	7.9 (7.2–18.5)	8.6 (7.3–13.2)	0.49
aPTT (sec)	10.0–14.0	11.6 (9.8–24)	11.3 (10.8–19.2)	0.92
Fibrinogen (g/l)	1.3–3.0	2.08 (0.6–3.93)	2.41 (0.82–2.94)	0.69
Antithrombin (%)	108–128	122 (87–153)	129 (64–164)	0.69
Protein C (%)	76–119	66 (5–104)	72 (3–96)	0.80
Protein S (%)	74–161	87 (0–145)	113 (43–200)	0.32
D-dimers (µg/l)	0.02–0.67	0,24 (0.07–0.62)	0.29 (0.16–0.87)	0.23
Lactate (mmol/l)	0.4–2.2	**6.2 (1.9–9.7)**	**11.8 (7.5–16.2)**	**0.01**
CRP (mg/l)	0–11	5.4 (2.4–15.7)	4.7 (0.1–133.2)	0.84
WBC (10⁹/l)	5.48–13.74	14.1 (4.2–26.8)	10.4 (9.2–18.8)	1.0
Neutrophils (10⁹/l)	2.78–8.73	12.77 (3.01–22.1)	8.91 (7.24–17.11)	0.84
Lymphocytes (10⁹/l)	0.72–4.71	0.9 (0.65–1.47)	0.82 (0.48–1.24)	0.23
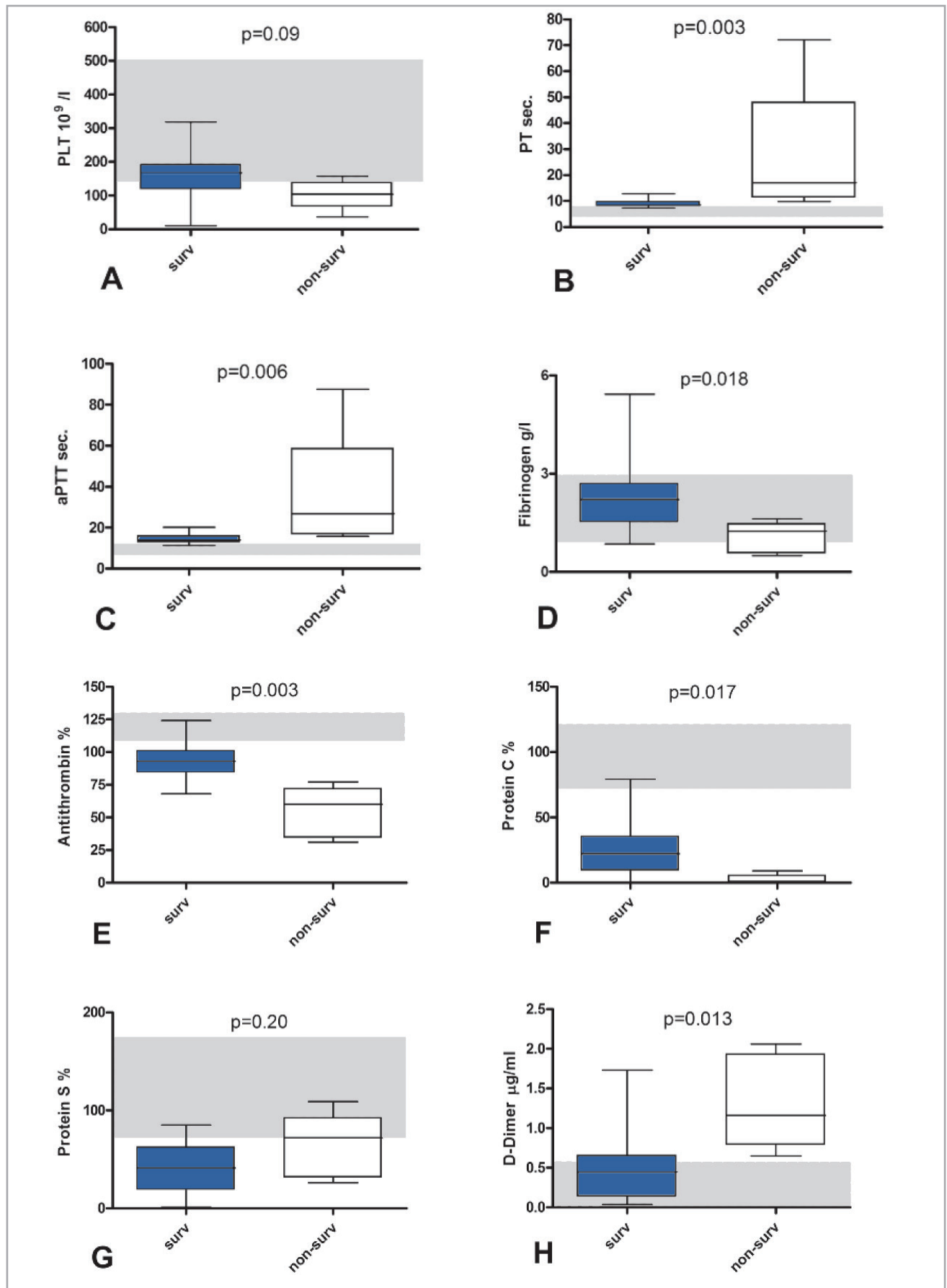
Fig. 1 A–H: Platelet count (PLT), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, antithrombin, protein C, protein S, and D-dimers in surviving dogs (surv) (n = 13) and non-surviving (non surv) (n = 5) dogs with gastric dilatation-volvulus at time point T1, day 1 after surgery. Results are shown as box and whisker diagrams. The central box represents the values from the lower to the upper quartile. The middle line is consistent with the median. The vertical line extends from the minimum to the maximum value. The grey area is consistent with the reference interval. Level of significance was set at ∝ = 0.05.

In contrast, significant differences were seen between survivors and non-survivors until discharge when evaluating results after surgery at T1. The median PT was significantly higher (17.1 sec, range 9.8–72.2 sec) in non-survivors compared to survivors (8.5 sec, range 7.3–12.8 sec) (see Fig. 1 B) and the median aPTT was significantly higher in non-survivors (median 26.9 sec, range 15.8–87.6 sec) compared to survivors (median 14.0 sec, range 11.4–20.3 sec) (see Fig. 1 C). The median fibrinogen concentration of non-survivors (median 1.24 g/l, range 0.5–1.63 g/l) was significantly lower than in survivors (median 2.21 g/l, range 0.85–5.43 g/l) (see Fig. 1 D). The median anticoagulant activity of PC was significantly lower in non-survivors (median 0%, range 0–9%) compared to survivors (median 22%, range 0–79%) (see Fig. 1 E). The median anticoagulant activity of AT was significantly lower in non-survivors (median 60%, range 31–77%) compared to survivors (median 93%, range 68–124%) (see Fig. 1 F). The median D-dimer concentration in non-survivors (median 1.16 µg/l, range 0.65–2.06 µg/l) was significantly higher than in survivors (median 0.64 µg/l, range 0.04–1.73 µg/l) (see Fig. 1 H). The median plasma lactate concentration was significantly lower in survivors than in non-survivors (p = 0.0078) (see Fig. 2 A). The CRP concentration was significantly lower in non-survivors than in survivors (p = 0.03) with non-survivors having a median CRP concentration of 50.6 mg/l (range 3.7–98.8 mg/l) versus 76.5 mg/l (range 51.6–153.6 mg/l) in survivors (see Fig. 2 B).
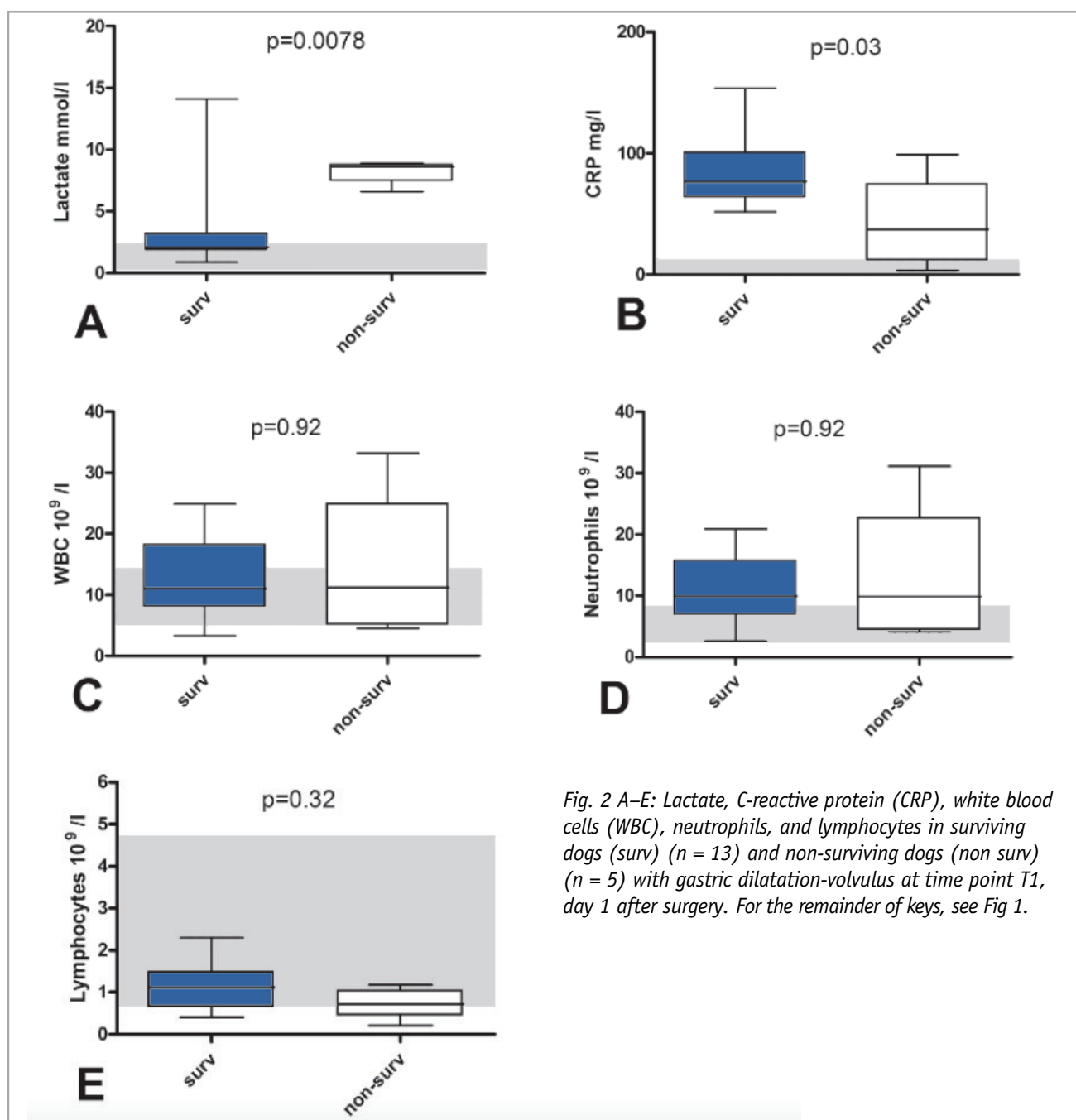

Fig. 2 A–E: Lactate, C-reactive protein (CRP), white blood cells (WBC), neutrophils, and lymphocytes in surviving dogs (surv) (n = 13) and non-surviving dogs (non surv) (n = 5) with gastric dilatation-volvulus at time point T1, day 1 after surgery. For the remainder of keys, see Fig 1.
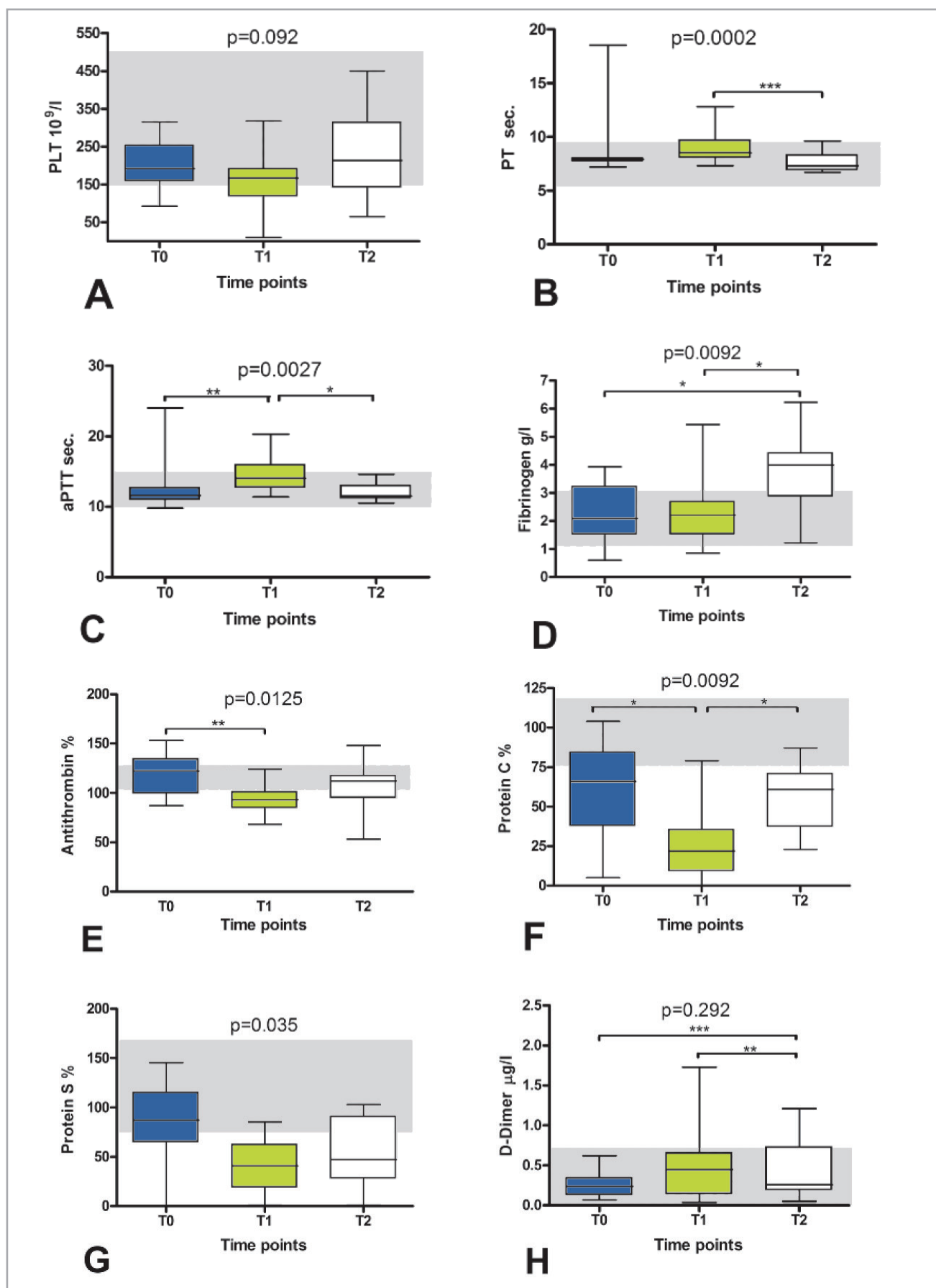
Fig. 3 A–H: PLT, PT, aPTT, fibrinogen, antithrombin, protein C, protein S, and D-dimers in surviving dogs with gastric dilatation volvulus (n = 13) at T0 (before surgery), T1 (day 1 after surgery) and T2 (day 3 after surgery).
* p < 0.05; ** p < 0.01; *** p < 0.001 are results of Dunn's post test. For the remainder of keys refer to Fig. 1.

Concerning coagulation response during the course of time in overall survivors, significant differences between time points T0, T1 and T2 were detected for PT (p = 0.0002), aPTT (p = 0.0027), fibrinogen (p = 0.0092), AT (p = 0.0125) and PC (p = 0.0092) (see Fig. 3 A–H). The median PLT count was at the lower end of the reference interval (see Fig. 3 A) with individual dogs showing thrombocytopenia. However, there was no significant change of the PLT count during the time evaluated time points. Median PT and aPTT were within the reference interval with a mild, but significant increase at T1, however, there was a broad inter-individual variation especially at T0. At T0 and T1, the median fibrinogen concentration was within the reference interval with

a range exceeding the lower and the upper range of the reference interval. At T2, the median fibrinogen concentration increased significantly above the upper limit of the reference interval and hyperfibrinogenaemia was seen in 9/13 (70%) of the dogs. The median AT activity was within the reference interval prior to surgery but decreased significantly below the reference interval directly after the operation (see Fig. 3 E). At the time of discharge, the median was within the reference interval again but there was a broad inter-individual variation. The median protein C activity was markedly below the lower limit of reference interval at all time points for the majority of dogs (see Fig. 3 F), however, there was a marked decrease directly after surgery at T1. A similar but less severe
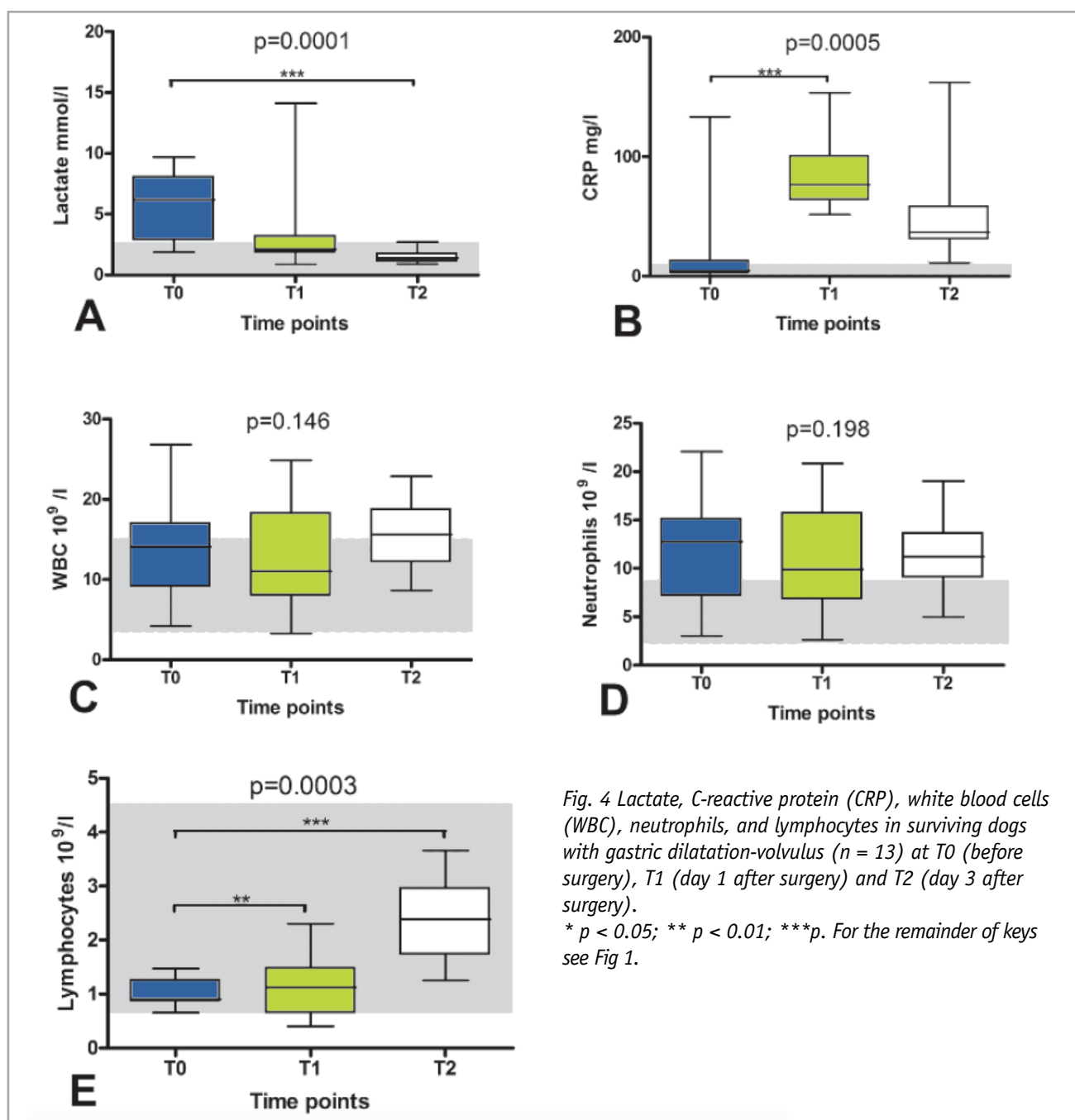

Fig. 4 Lactate, C-reactive protein (CRP), white blood cells (WBC), neutrophils, and lymphocytes in surviving dogs with gastric dilatation-volvulus (n = 13) at T0 (before surgery), T1 (day 1 after surgery) and T2 (day 3 after surgery).
* p < 0.05; ** p < 0.01; ***p. For the remainder of keys see Fig 1.

alteration was seen for the protein S activity (see Fig. 3 G). Median D-dimer concentration was within the reference interval at all time points, however, there was a significant increase after surgery until discharge with individual dogs having severely increased values.

Lactate plasma concentration changed significantly (p = 0.0001) during time points. At T0, the lactate plasma concentration was increased above the upper reference interval (2.2 mmol/l) in 85% (11/13) of the surviving dogs at T0, in 38% (5/13) at T1 and still in 1/13 dogs at the time of discharge. The median lactate plasma concentration at T0 was significantly higher when compared to T2 (see Fig. 4 A). Concerning the inflammatory response, a significant increase of the median CRP concentration was noticed at T1 in contrast to T0 (see Fig. 4 B). Median WBCs (see Fig. 4 C) were at the upper limit of the reference interval and increased at the time of discharge, however, the finding was not significant. Median neutrophil count (see Fig. 4 D) was above the upper limit of the reference interval and did not change significantly during the course of the study. The median lymphocyte count was initially at the lower end of the reference interval with individual dogs showing a lymphopenia but increased significantly (p = 0.0003) from T0 to T1 and T2 respectively (Fig. 4E).

Discussion

The present study clearly demonstrated severe coagulation disorders especially regarding endogenous anticoagulants in dogs with GDV. The lactate plasma concentration was the only prognostic factor for overall survival. In contrast coagulation and inflammatory response was of no prognostic value for predicting overall survival in dogs with GDV. The delayed response of the coagulation system and the inflammatory reaction to GDV noted in our study may explain the marked inter-individual variation of coagulation and inflammation markers and consequently their absent utility as markers for predicting overall survival.

The activation of coagulation can be explained by the pathophysiologic mechanisms of GDV leading to hypovolemic, obstructive shock with hypoperfusion, ischemia and tissue necrosis, all known to activate coagulation response. Additionally, endotoxins have been shown to activate coagulation[5,29].

Experimental endotoxaemia-associated coagulation abnormalities in healthy dogs demonstrated an early rise in D-dimers followed by a marked decrease in protein C and protein S, and AT in the first 24 hours[17]. Treatment of GDV includes reperfusion with massive release of endotoxins additionally stimulating the coagulation system by initiating an inflammatory process[5,29]. Depending on the duration and severity of activation of the inflammatory process and subsequently activation of the coagulation system, consumption of coagulation factors and naturally anticoagulants will finally result in the development of DIC[2]. Overt DIC was diagnosed postoperatively in nearly one third of the dogs comparable to results of Millis et al.[33] (40%) and Abbrederis[1] (38.5%). The increase of fibrinogen plasma concentration at the time of discharge most likely reflects an activation of coagulation due to an inflammatory reaction following GDV and surgery as well as a decreased consumption. It is well known that fibrinogen as a positive acute phase protein shows a less severe and delayed response in contrast to CRP[12,34].

Especially regarding the activities of endogenous anticoagulants, many dogs still showed marked abnormalities until the day of discharge. Similar findings have been observed in dogs with naturally occurring sepsis showing that PC- and AT activities changed significantly over time in dogs with sepsis and both were likely related to survival[15]. In accordance with results recognized in people[20] and dogs with SIRS[5], a less pronounced decrease in protein S compared to AT and protein C was visible. This finding may be explained by the particular sensitivity of the protein C pathway to down regulation by inflammatory processes[18]. The overall decrease in endogenous anticoagulants in the present study once more shows the enormous impact of GDV on activating coagulation response with emphasis on consumption of endogenous anticoagulants. Additional studies are needed to determine whether these changes of endogenous anticoagulants correlate with the severity of GDV.

In accordance with others[7,16,25,40] we found that plasma lactate concentration is a prognostic factor before treatment of dogs with GDV. Furthermore, overall survivors showed a significant decrease of the median lactate plasma concentration during treatment. Serial measurements of plasma lactate have already been proven to be a prognostic marker in critical ill patients, i.e. dogs with GDV[36,38,40]. The inflammatory biomarkers that were assessed showed no significant differences between overall survivors and non-survivors. CRP concentration as a marker for the acute phase reaction was characterized by a significant

increase after surgery and a decrease on discharge, which has been described earlier[22,37]. In contrast to other inflammatory variables, CRP is known to be an early marker for inflammatory processes especially after surgery and is related to postoperative complications[28]. Nevertheless, our study clearly shows that even changes of CRP are not rapid enough to be a preoperative prognostic marker. Prior to and after surgery, lymphocyte count was at the lower end of the reference interval or mild lymphopenia was present, which could be in response to endogenous corticosteroid release due to stress, pain, and inflammation[13,23].

The small number of dogs clearly is a limitation of the study. Sample collection and surgery were not performed by the same veterinarian, solely method and chronology of this study were predefined. Duration of anaesthesia was not constant during the study. However, anaesthesia has been stated to have no influence on outcome in dogs with GDV[32]. Whether time lapse until treatment of dogs with GDV influenced haemostatic abnormalities in the current study was not investigated. It certainly seems to influence outcome as reported previously[11]. Regarding fluid therapy, there seems to be a certain impact of isotonic as well as hypertonic saline solutions on the coagulation cascade, i.e. isotonic saline solution can cause either a hypercoagulable (lower dilutions) or hypocoagulable (higher dilutions) derangement. Hypertonic saline solution can lead to more pronounced coagulation abnormalities (hypocoagulable derangements)[26]. Whether fluid composition during surgery influenced coagulation variables in the pre sent study remains unclear. However, after T1 none of the dogs received hypertonic saline solution. Therefore, the coagulation abnormalities of endogenous anticoagulants seem to be disease-related.

Overall, we concluded that only plasma lactate concentration was of preoperative prognostic value. However, in dogs surviving surgery, coagulation variables especially the endogenous anticoagulants and CRP reflecting inflammation can be used as additional prognostic markers for the survival until discharge. As marked abnormalities of coagulation variables are still present at the time of discharge, future studies are necessary to elucidate the time needed for normalization of the coagulation response and the inflammatory reaction respectively in dogs with GDV.

Conflict of interest
The authors confirm not to have any conflict of interest in the present study.

Conclusion for practice

The present study emphasizes severe haemostatic abnormalities in dogs with GDV especially regarding their endogenous anticoagulant activities. Before surgery, coagulation variables are of no prognostic value, however, after surgery, they are associated with survival until discharge. Dogs dying after surgery showed significantly longer coagulation times and higher D-dimer concentrations as well as lower fibrinogen concentrations and activities of PC and AT compared to survivors until discharge. As disorders of haemostasis in dogs with GDV can worsen prognosis, serial measurements of these variables in the course of the disease might improve outcome. Measurement of plasma lactate concentration is the only preoperative variable of prognostic value for overall survival.

References

1. Abbrederis N. Validierung von Laborparametern zur Diagnose einer DIC beim Hund. Inaugural-Dissertation, Ludwig-Maximilians-Universität, München 2005.
2. Bauer N, Moritz A. Koagulopathien bei Hund und Katze. *Tierärztl Prax* 2009; 36 (K): 235–246.
3. Bauer N, Eralp O, Moritz A. Reference intervals and method optimization for variables reflecting hypo- and hypercoagulatory states in dogs using the STA Compact automated analyser. *J Vet Diagn Invest* 2009; 21: 803–814.
4. Bauer N, Er E, Moritz A. Influence of blood sampling techniques on platelet function and coagulation parameters in dogs. *Am J Vet Res* 2011; 72: 64–72.
5. Bauer N, Moritz A. Coagulation response in dogs with and without systemic inflammatory response syndrome – Preliminary results. *Res Vet Sci* 2013; 94: 122–133.
6. Beck JJ, Staatz AJ, Pelsue DH, Kudnig ST, MacPhail CM, Seim HB, Monnet E. Risk factors associated with short-term outcome and development of perioperative complications in dogs undergoing surgery because of gastric dilatation-volvulus: 166 cases (1992–2003). *J Am Vet Med Assoc* 2006; 229: 1934–1939.
7. Beer KAS, Syring RS, Dobratz KJ. Evaluation of plasma lactate concentration and base excess at the time of hospital admission as predictors of gastric necrosis and outcome and correlation between those variables in dogs with gastric dilatation-volvulus: 78 cases (2004–2009) *J Am Vet Med Assoc* 2013; 242: 54–58.
8. Bentley AM, Mayhew PD, Culp WTN, Otto CM. Alterations in the haemostatic profiles of dogs with naturally occurring septic peritonitis. *J Vet Emerg Crit Care* 2013; 23: 14–22.

10. Bruhn HD, Schambeck CM, Hach-Wunderle V. Häufige Fehlursachen. In: Hämostaseologie für die Praxis. Bruhn HD, Schambeck CM, Hach-Wunderle V, eds. Stuttgart: Schattauer 2007; 29.

11. Buber T, Saragusty JE, Ranen A, Epstein T, Bdolah-Abram, Bruchim Y. Evaluation of lidocaine treatment and risk factors for death associated with gastric dilatation and volvulus in dogs: 112 cases (1997–2005). *J Am Vet Med Assoc* 2007; 230: 1334–1339.

12. Ceron JJ, Eckershall PD, Martinez-Subiela S. Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Vet Clin Pathol* 2005; 34: 85–99.

13. Cotter SM. Patterns of Change in Lymphocytes Numbers. In: Quick Look Series in Veterinary Medicine, Hematology. Cotter SM, ed. Jackson: Tenton NewMedia 2001; 75.

14. de Laforcade AM, Freeman LM, Shaw SP, Brook MB, Rozanski EA, Rush JE. Haemostatic changes in dogs with naturally occurring sepsis. *J Vet Intern Med* 2003; 17: 674–679.

15. de Laforcade AM, Rozanski EA, Freeman LM, Li W. Serial evaluation of protein C and antithrombin in dogs with sepsis. *J Vet Intern Med* 2008; 22: 26–30.

16. de Papp E, Drobatz KJ, Hughes D. Plasma lactate concentration as a predictor of gastric necrosis and survival among dogs with gastric dilatation-volvulus: 102 cases (1995–1998). *J Am Vet Med Assoc* 1999; 215: 49–52.

17. Eralp O, Yilmaz Z, Failing, K, Moritz A, Bauer N. Effect of experimental endotoxemia on thrombelastography parameters, secondary and tertiary haemostasis in dogs. *J Vet Intern Med* 2011; 25: 524–531.

18. Esmon CT. The impact of the inflammatory response on coagulation. *Thromb Res* 2004; 114: 321–327.

19. Fischer CJ, Yan SB. Protein C levels as a prognostic indicator of outcome in sepsis and related diseases. *Crit Care Med* 2000; 28: 49–56.

20. Fourrier F, Chopin C, Goudemand J, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation. Compared patterns of antithrombin III, protein C, and protein S deficiencies. *Chest* 1992; 101: 816–823.

21. Franchini M, Veneri P, Lippi G. Inflammation and haemostasis: a bidirectional interaction. *Clin Lab* 2007; 53: 63–67.

22. Galezowski AM, Snead EC, Kidney BA, et al. C-reactive protein as a prognostic indicator in dogs with acute abdomen syndrome. *J Vet Diagn Invest* 2010; 22: 395–401.

23. Glickman LT, Glickman NW, Perez CM, Schellenberg DB, Lantz GC. Analysis of risk factors for gastric dilatation and dilatation-volvulus in dogs. *J Am Vet Med Assoc* 1994; 204: 1465–1471.

24. Goldmann F, Bauer N, Moritz A. Comparison of the ProCyte Dx analyser with the ADVIA 2120 and the manual differential for validation of equine and bovine hemograms. *Comp Clin Pathol* 2013; 22: 855–868.

25. Green TI, Tonozzi CC, Kirby R, Rudloff E. Evaluation of initial plasma lactate values as a predictor of gastric necrosis and initial and subsequent plasma lactate values as a predictor of survival in dogs with gastric dilatationvolvulus: 84 dogs (2003–2007). *J Vet Emerg Crit Care* (San Antonio) 2011; 21: 36–44.

26. Kaczynski J, Wilczynska W, Hilton J, Fligelstone L. Impact of crystalloids and colloids on coagulation cascade during trauma resuscitation – a literature review. *Emerg Med Health Care* 2013; 1: 1–6.

27. Klenner S, Bauer N, Moritz A. Evaluation of three human immunoturbidimetric assays for the detection of C-reactive protein in dogs. *J Vet Diagn Invest* 2010; 22: 544–552.

28. Knapp A, Schrödl W, Grevel V, Oechtering G. Konzentration des C-reaktiven Proteins (CRP) im Blutserum bei Hunden mit postoperativen Wundheilungsstörungen. *Tierärztl Prax* 2009; 37 (K): 21–27.

29. Levi M, de Jonge E, van der Poll T. Sepsis and disseminated intravascular coagulation. *J Thromb Thrombolysis* 2003; 16: 43–47.

30. Levi M, van der Poll T. The role of endogenous anticoagulants in the pathogenesis and management of systemic activation of coagulation and inflammation in critically ill patients. *Semin Thromb Hemost* 2008; 34: 459–468.

31. Lorente JA, Garcia-Frade LJ, Landin L, et al. Time course of haemostatic abnormalities in sepsis and its relation to outcome. *Chest* 1993; 17: 391–398.

32. Mackenzie G, Barnhart M, Kennedy S, DeHoff W, Schertel E. A retrospective study of factors influencing survival following surgery for gastric dilatation-volvulus syndrome in 306 dogs. *J Am Anim Hosp* Assoc 2010; 46: 97–102.

33. Millis DL, Hauptman JG, Fulton Jr RB. Abnormal haemostatic profiles and gastric necrosis in canine gastric dilatation-volvulus. *Vet Surg* 1993; 22: 93–97.

34. Monreal L. D-Dimer as a new test for the diagnosis of DIC and thromboembolic disease. *J Vet Intern Med* 2003; 17: 757–759.

35. Neumaier M, Scherer MA, Von Grumppenberg S. Das C-reaktive Protein bei tiefer Wundinfektion. *Akt Traumatol* 2000; 30: 69–99.

36. Nguyen BH, Rivers EP, Knoblich BP, Jacobson GMS, Muzzin ABS, Ressler JA, Tomlanovich MC. Early lactate clearance is associated with improved outcome in severe sepsis and septic shock. *J Crit Care Med* 2004; 32: 1637–1642.

37. Otabe K, Ito T, Sugimoto T, Yamamoto S. C-reactive protein (CRP) measurement in canine serum following experimentally-induced acute gastric mucosal injury. *Lab Anim* 2000; 34: 434–438.

38. Stevenson CK, Kidney BA, Duke T, Snead CR, Mainar-Jaime RC, Jackson ML. Serial blood lactate concentrations in systemically ill dogs. *Vet Clin Pathol* 2007; 36: 234–239.

39. Wiinberg B, Jensen AL, Johansson PI, Rozanski E, Tranholm M, Kristensen AT. Thromboelastographic evaluation of haemostatic function in dogs with disseminated intravascular coagulation. *J Vet Intern Med* 2008; 22: 357–365.

40. Zacher LA, Berg J, Shaw SP, Kudej RK. Association between outcome and changes in plasma lactate concentration during presurgical treatment in dogs with gastric dilatation-volvulus: 64 cases (2002–2008). *J Am Vet Med Assoc* 2010; 236: 892–897.

Viewpoint

Lyssa lingualis: debunking the myth of the 'tongue worm'

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SUMMARY

The lyssa is an anatomic structure located in the median plane of the rostroventral surface of the tongue in carnivores. Although no great importance is given to this structure, the myth that there is a 'worm' under the tongue that is responsible for rabies in dogs was first described in Greek civilization. Nowadays, the myth that excision of this 'worm' can cure animals with rabies, distemper and parvovirus still remains among dog owners in several countries, including Portugal. This work aims to describe the lyssa and demonstrate that it is not a 'worm'. We also intend to advise owners to avoid unnecessary suffering of the animals when excising this so-called 'worm'. A tongue was obtained from a cadaver of a female dog. Histologically, the lyssa was encapsulated in a dense sheath of connective tissue, filled with adipose tissue and striated muscle, demonstrating that it is not a worm. Although the lyssa is frequently ignored during the clinical examination of companion animals, vets should be aware of the practice of lyssa excision in certain parts of the world and advise owners that it is a non-therapeutic procedure, prohibited and punishable by law.

Keywords: anatomy, dog, lyssa, microscopy, tongue 'worm'

Introduction

The lyssa is an anatomic structure derived from the neuroectoderm. It may be easily felt by palpation in the median plane of ventral surface of the tongue of carnivores. The lyssa extends from almost the tip to the root of the tongue (short of the lingual frenulum), not reaching the hyoid bone[1,2].

In Greek mythology, Lyssa was the goddess of madness, rage, frenzy and responsible for the occurrence of rabies in animals[3,4]. According to historical data, the first veterinary procedures of dentistry were performed by the Romans in dogs with rabies in order to remove the lyssa and 'cure' them. According to legend, "it must be carried three times about the fire in order to be effective when given to someone bitten by a rabid dog. Take the worm from under a mad dog's tongue, leave them around a fig tree, then give them to him that hath been rent."[5]

This myth has passed from generation to generation up until the present day. Nowadays, the idea that the lyssa is a 'worm' under the tongue that causes disease in dogs, namely parvoviruses, distemper or rabies, still remains in several countries, including in Portugal, where the myth is particularly persistent among dog owners. The aim of this paper is to describe the lyssa and demonstrate that it is not a 'worm'. We also intend to inform pet owners of the unnecessary suffering inflected on animals during procedures employed when excising this so-called "worm".

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Materials and Methods

The tongue was obtained from the cadaver of a female, 10-week-old dog of approximately 8kg body weight. Because of an intussusception, the owner's consent was obtained. The animal was humanely euthanised by the intravenous injection of pentobarbital sodium (140 mg/kg, Euthasol®, Esteve, Portugal). Immediately after its excision, the tongue was immersed in 10% buffered formalin for 24 hours. After this, the tongue was cut in transverse sections, processed and embedded in paraffin. Then, 5 μm sections were cut and routinely stained with haematoxylin and eosin (H&E). The histological slides were observed under a light microscope (DM500, Leica Microsystems, Germany) and photographed using the attached camera (ICC50, Leica Microsystems, Germany).

Results

Macroscopic findings

The lyssa was identified as a rod-shaped fibrous structure, white in colour, located along the median plane on the ventral surface of the free portion of the tongue. It extended from almost the tip of the tongue to the root of the tongue (to the lingual frenulum), not reaching the hyoid bone. It measured 4.9 cm long (Fig. 1 A and B).

Microscopic findings

The lyssa was in the submucosa of the tongue. Histologically, the lyssa was encapsulated in a dense sheath of connective tissue, filled with adipose tissue and striated muscle (dorsal to the adipose tissue) (Fig. 1 C and D).
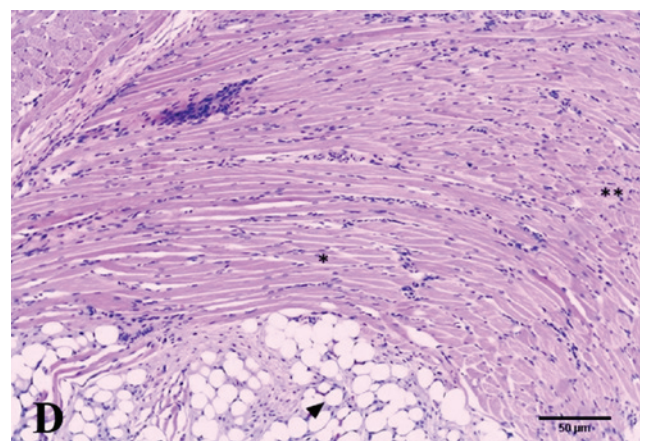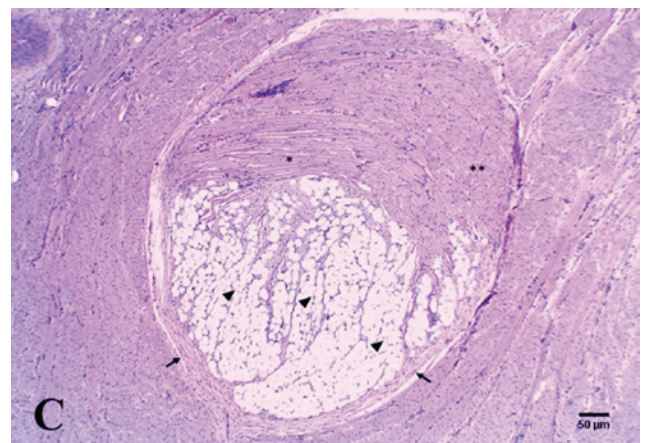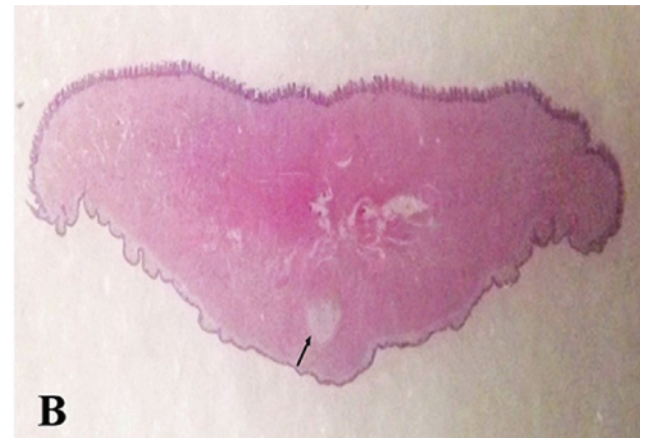
Figure 1. Macroscopic (A and B) and microscopic appearance (C and D) of the lyssa in a dog.

(A) and (B).
The lyssa is a fibrous cord, white in colour, located in the ventral free portion of the tongue (arrow). It is cranial to the lingual frenulum (asterisks).

(C) and (D).
Histologically, the lyssa is composed of striated muscle (transverse fibres* and longitudinal fibres**), adipose tissue (arrowheads) and surrounded by a dense sheath of connective tissue (arrows). (C) H&E, magnification of 4×; (D) H&E, magnification of 10×.

Discussion and Conclusion

Although the function of the lyssa is not understood and clinicians do not give great importance to this anatomic structure in clinical practice, it has been considered by owners as a 'worm' responsible for rabies, distemper and parvovirus. This issue was first recorded in Greek civilization, where the goddess Lyssa was believed responsible for the occurrence of rabies in dogs. Rabies is caused by a virus belonging to the genus Lyssavirus, which derives from the Greek word 'lyssa'[6,7].

Taking into account this ancestral knowledge, many people still believe that the removal of this 'worm' can improve the animal's health and cure sick animals. Consequently, this structure is often removed using unacceptable techniques. The animals are not sedated or anaesthetized, an incision is made in the midline ventral surface of the tongue using a blade, and the lyssa 'worm' is excised. After its removal, the lyssa can exhibit a weak contractile movement, suggesting that it is a worm. However, it was confirmed by histological studies that the lyssa is an anatomical structure surrounded by connective tissues and filled with striated muscle and adipose tissue. Although chondroid cells were not found in our study, they were observed in previous studies[8,9]. Similarly, in a study performed by Besoluk and collaborators,[10] chondroid cells were not found in the lyssa of dogs. As the lyssa is not a worm, its removal cannot be considered a medical treatment, and is more akin to an act of witchcraft, causing considerable animal suffering. It is therefore imperative to inform animal owners that there is no 'worm' under the tongue and the removal of the lyssa will certainly not help to treat sick animals. It is also important to warn them of the unnecessary suffering caused to the animals during the process of excision. According to Portuguese (Decree-Law nº 260/2012) and European Legislation (European Convention on Welfare Companion Animals) any act that causes pain or lesions, or causes an animal's death is forbidden. Recently, Law nº 69/2014 added the criminalization of these acts and Law nº 110/2015 implemented additional punishments, namely being banned from keeping animals for a period of up to five years[11-14].

Although the lyssa is frequently ignored during clinical examination of companion animals, veterinary surgeons and nurses should pay attention to it to detect any pathologic structures there (intermandibular and dermoid cysts found the mouth and tongue), or on the lingual frenulum (partial or complete ankyloglossia and sublingual frenectomy)[15,16]. They should also be aware of the practice of excision of the lyssa and advise owners that it is a non-therapeutic procedure, prohibited and punishable by law.

Acknowledgments

Conflict of interests:

None to declare.

References

1. Barone R. Anatomie Comparée des Mammifères Domestiques.ed. Paris: Vigot; 1996. pp.
2. International Committee on Veterinary Gross Anatomical Nomenclature. Nomina Anatomica Veterinaria. 5th edition ed. Germany, USA, Belgium, Japan: Editorial Committee Hannover, Columbia, Ghent, Sapporo; 2012.
3. Vellacott P. Herakles by Euripides. Published Penguin Books; 1963.
4. Grant M. The Myths of Hyginus.ed. University of Kansas Press; 1960.
5. Harvey C. The history of veterinary dentistry part one: from the earliest record to the end of the 18th century. *J Vet Dent* 1994; 11: 135-139.
6. International Committee on Taxonomy of Viruses (ICTV): Rhabdoviridae; 2016.
7. Murphy F, Bibbs,E, Hozinek,M, Studdert,M. Veterinary Virology. 3rd ed. San Diego, USA: Academic Press; 1999. pp. 429-445.
8. Budras K, Fricke,W, McCarthy,P. Anatomy of the dog. 3rd ed. London: Mosby-Wolfe; 2004. pp.
9. Frewein H, Vollmerhaus,B. Anatomie von Hund und Katze.ed. Berlin: Blackwell Wissenschafts-Verlag; 1994.
10. Besoluk K, Eken E, Sur E. Morphological studies on lyssa in cats and dogs. *Vet Med* 2006; 51: 485-489.
11. Decreto-Lei nº 110/2015 d2da. Quadro de penas acessórias aplicáveis aos crimes contra animais de companhia. *Diário da República* - Portugal 2015.
12. Lei nº 69/2014 d2da. Criminalização dos maus tratos a animais de companhia, proteção dos animais, alargamento dos direitos das associações zoófilas. *Diário da República* - Portugal 2014.
13. European Convention for the Protection of Pet Animals. Animal welfare in the light of current veterinary scientific knowledge. Council of Europe 1992.

14. Decreto-Lei nº 260/2012 d1dd. Normas legais tendentes a pôr em aplicação em Portugal a Convenção Europeia para a Proteção dos Animais de Companhia. *Diário da República* - Portugal 2012.
15. Eisenmenger E, Zetner, K. *Veterinary Dentistry*. ed. Philadelphia: Lea & Febiger; 1985. pp. 140.
16. Liptak J, Canfield P, Hunt G. Dermoid cyst in the tongue of a dog. *Aust Vet J* 2000; 78: 160-161.