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2016, vol. 85, nr. 6

INHOUD

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Fysiotherapie bij de huisdieren bestaat al verscheidene jaren. Toch is dit bij een groot aantal dierenartsen nog onbekend terrein, terwijl dieren, net als mensen, baat hebben bij een gecontroleerde en geassisteerde revalidatie. Niemand stelt zich vragen bij humane kinesitherapie. Hopelijk zal in de komende jaren ook niemand nog raar opkijken wanneer de dierenarts een aangepast revalidatieschema voor zijn favoriete huisdier opstelt (cf. pg. 323)

Tekst: Yves Samoy

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Physiotherapy in small animal medicine

Fysiotherapie bij kleine huisdieren

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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 already 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.

SAMENVATTING

De voordelen van fysiotherapie zijn reeds lang bekend in de humane geneeskunde. Ook in de diergeneeskunde wordt de therapie reeds enkele decennia toegepast, maar het is pas vrij recentelijk dat er ook wetenschappelijk onderzoek naar verricht wordt. Het doel van dit artikel is een overzicht te geven van de verschillende fysiotherapeutische mogelijkheden en technieken in de diergeneeskunde en deze te correleren aan de bevindingen die te vinden zijn in de literatuur.

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 physiotherapeutic 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). 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 lacks in veterinary medicine (Landrum et al., 2008; Zusman, 2010). Although the principles are similar, straight forward interspecies extrapolation should be treated carefully. In one study, massage was actively incorporated in the physiotherapeutic protocol of dogs with degenerative myolopathy, besides active and passive exercises and hydrotherapy (Kathmann et al., 2006). This report demonstrates that intensive physiotherapeutic treatment may prolong the life expectancy by 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 are 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 gives 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 reliefs 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 more when using icepacks (all or not combined with bandaging), than with the use of bandaging alone (Rexing et al., 2010). In the other 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 ac-

tual healing effect of heat in small animal medicine can be found to date (Millard et al., 2013). Besides the purely healing effect, heating soft tissues 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 the 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.

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). 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.
- Substania gellatinosa (SG) cells inhibit the pain signal to the brain.

By overstimulating 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).

Figure 1. Neurotrac® TENS/NMES device.

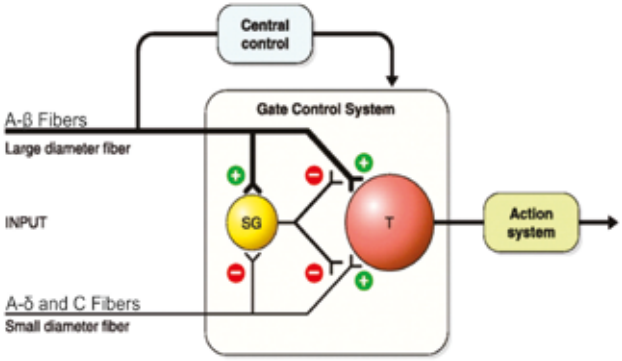

Figure 2. Gate control theory (Adapted from Zeilhofer et al., 2012)

Figure 3. The use of NMES on the front leg of a dog.

TENS has been demonstrated efficient in orthopedic 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)

NMES indicates that 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).

NMES has been used in a series of orthopedic 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.

Soundwaves

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 soundwave corresponds with the amplitude and is usually measured in Watt per cm² (Bockstahler et al., 2004).

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

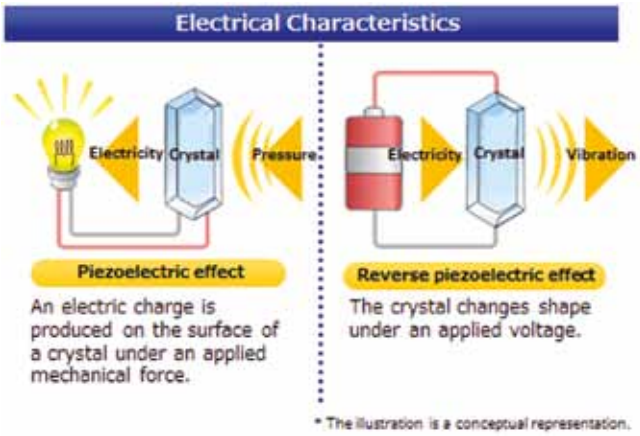

Figure 4. Mechanism of a piezoelectric crystal (Source: Global Epson).

Figure 5. Use of ultrasound on the stifle of a dog.

used to 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, 3MH 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 would 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 soundwaves 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 not available. 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 hemoglobin, 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.

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).

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 enkephalins (Millis et al., 2005; Mil-

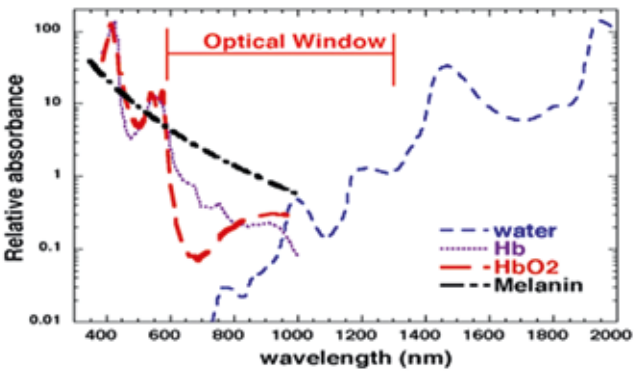

Figure 6. Optimal wavelength in LASER treatment. Hb= Hemoglobin; HbO2= Oxygenated Hemoglobin (Source: www.spie.com)

Figure 7. Laser therapy in a dog with chronic elbow arthritis.

lis 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 myelinization 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 are 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

Figure 8. Assisted walking device used in a dog with surgically treated Wobbler's syndrome.

Figure 9. Balance board exercise.

Figure 10. Cavaletti rail.

weight bearing and proprioceptive training (Bockstahler et al., 2004). An example is balance board exercises (Figure 9).

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 edema 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

Figure 11. Underwater treadmill for dogs.

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. 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).

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 orthopedic 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.

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The efficacy of chloroquine treatment of *Giardia duodenalis* infection in calves

De werkzaamheid van chloroquinebehandeling van Giardia duodenalis-*infectie bij kalveren*

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ABSTRACT

The purpose of the present study was to evaluate the effect of chloroquine treatment on cyst excretion in calves naturally infected with *Giardia duodenalis*. The calves were randomly assigned into two groups based on placebo (group I, n=7 untreated control calves) or treatment (group II, n=7 calves treated orally with 2.5 mg/kg chloroquine twice daily for five consecutive days). The *G. duodenalis* isolates were identified by molecular characterization with β-giardin nested PCR and gene sequence analysis as assemblage A3. Cyst excretion was determined on days 0, 3, 7 and 10, before and after treatment. Geometric means of the number of excreted cysts did not change significantly in the control group during the trial. The reduction in cyst excretion after chloroquine treatment was 99% on day 3 and 100% on days 7 and 10. Chloroquine treatment is most probatly practically applicable, relatively inexpensive and highly effective against giardiosis in calves.

SAMENVATTING

Het doel van deze studie was om het effect van chloroquinebehandeling te evalueren op de cysteexcretie van kalveren met een natuurlijke infectie met *Giardia duodenalis*. De kalveren werden willekeurig ingedeeld in twee groepen op basis van placebo (groep I, n = 7 onbehandelde controlekalveren) of behandeling (groep II, n = 7 kalveren behandeld met chloroquine). De behandeling gebeurde door orale toediening, tweemaal daags gedurende vijf opeenvolgende dagen aan een dosis van 2,5 mg/kg lichaamsgewicht. Door middel van moleculaire typering met ß-giardin geneste PCR en gensequentieanalyse werden de *G. duodenalis*-isolaten geïdentificeerd als behorende tot het A3-assemblage. Cysteuitscheiding werd bepaald op dag 0, 3, 7 en 10, zowel voor als na de behandeling. De geometrische gemiddelden van het aantal uitgescheiden cysten waren niet significant veranderd tijdens het experiment in de controlegroep. De cyste-excretie was met 99% verminderd op dag 3 en met 100% op dag 7 en 10 bij de behandelde kalveren. Chloroquine is dus mogelijk een praktisch haalbare, relatief goedkope en zeer effectieve behandeling tegen giardiose bij kalveren.

INTRODUCTION

Giardiosis is an ubiquitous, intestinal protozoal infection distributed worldwide within the vast majority of domestic/wild mammals and humans. Transmission of *Giardia duodenalis* infection occurs via cysts, which are excreted in the feces of infected humans and animals (O'Handley and Olson, 2006). Livestock infection is also common and has been reported in 9%–73% of fecal samples from cattle, with higher rates of infection in calves. Farm prevalence rates might rise up to 100% (Geurden et al., 2010). Giardiosis in livestock animals is associated with a high morbidity, which may result in significant production losses (Aloisio et al., 2006; Geurden et al., 2010; Sweeny et al., 2011). Typically, chronic or reoccurring infections exist in calves and cyst excretion might continue for months. Clinical signs include diarrhea, weight loss,

lethargy and poor condition of the calves, but subclinical infections are common (Geurden et al., 2006).

Ruminants are most commonly infected by *G. duodenalis* of genetic assemblage E, which is considered to be livestock specific (O'Handley and Olson, 2006). Indeed, mono or mixed infections with genetic assemblage A (Geurden et al., 2008; Feng et al., 2008; Mark-Carew et al., 2012) or infection with genetic assemblage B (Coklin et al., 2007) have been reported. Thus, calves have been thought to have the potential to serve as a reservoir for human giardiosis (O'Handley et al., 2000; Trout et al., 2007; Uehlinger et al., 2007; Winkworth et al., 2008). However, recent molecular studies with multi-locus genotyping of assemblages A and B have shown that animals do not share identical genotypes with humans in most cases, which provides limited support of their role in zoonotic transmission (Sprong et al., 2009; Lebbad et al., 2010; Caccio, 2015).

The high prevalence rates, risk of production losses and zoonotic risk warrant suitable treatment of *G. duodenalis* infections in ruminants. Currently, there are no Food and Drug Administration (FDA)/ European Medicines Agency (EMA) approved drugs available for the treatment of giardiasis in cattle. The traditional treatment of giardiosis in calves consists of fenbendazole (Xiao et al., 1996; O'Handley et al., 1997; O'Handley et al., 2000; Garossino et al., 2001; O'Handley et al., 2001; Geurden et al., 2010), albendazole (Xiao et al., 1996; Ragbetli et al., 2014) or paramomycin (Geurden et al., 2006). Uehlinger et al. (2007) evaluated the efficacy of vaccination in the prevention of *G. duodenalis* infection in calves, but no differences were found between vaccinated and non-vaccinated calves in occurrence of giardiosis or cyst shedding.

The literature regarding the efficacy of treatment against giardiosis in ruminants is limited to the aforementioned studies. Taking into account cases refractory to traditional treatment in humans (Nash et al., 2001; Nabarro et al., 2015), there is still a need to establish reasonably priced and novel therapeutic protocols. Benzimidazoles are quite reasonably priced. Moreover, these compounds are already registered for the use in cattle as anthelmintics (O'Handley et al., 1997; O'Handley et al. 2001), which is not the case for chloroquine. Although fenbendazole has shown to be 100% effective and eliminated cysts from the feces within 6 days in calves, reinfection was observed in some of the treated calves within four weeks of trial (O'Handley et al., 1997). Chloroquine, a 4-aminoquinoline compound used for treatment of malaria, has recently been re-identified as an old drug with new perspective against giardiosis in humans (Escobedo et al., 2015).

Therefore, the purpose of this study was to evaluate the effect of chloroquine treatment on the cyst excretion in calves naturally infected with *G. duodenalis*.

MATERIALS AND METHODS

Animals, housing and treatment applications

A total of fourteen Holstein Friesian calves of one to three months old and of both sexes were enrolled into the study. A commercially available solid phase immunochromatographic assay (Anigen Rapid Bovid-5 Ag test Kit, Bionote, Korea) was used for the rapid, qualitative detection of Rotavirus, Coronavirus, *Escherichia coli* K99, *Cryptosporidium parvum* and *G. duodenalis* in calf feces. Initial diagnosis of mono infection with *G. duodenalis* was confirmed microscopically by detection of cysts in the fecal samples. The samples were withdrawn at different times of the year from several farms.

Calves naturally mono-infected with *G. duodenalis* were selected and randomly assigned into two groups that received either a placebo (group I, n=7 untreated control calves) or treatment (group II, n=7 calves treated with chloroquine). Enrolled calves were weighed prior to drug application via a cattle weighing scale having a calibration of 1 kg. The weighing was performed by the animal keepers not involved and informed within the trial. The calves in the treatment group received chloroquine (Kutlu tablet® 250 mg, Keymen, Turkey) twice daily at a dosage of approximately 2.5 mg/kg body weight orally (by the investigator applied directly into the mouth, followed by 10 ml water) for five consecutive days, whereas the calves in the control group received a placebo. The treatment dose was rounded off to the nearest weight. Calves weighing up to 50 kg received 125 mg compound, i.e. half a tablet, while calves weighing 50-100 kg received one tablet. The placebo included water with an equivalent volume as in the treatment group. Systematic clinical examinations were carried out and fecal samples were collected on days 0, 3, 7 and 10 after the first administration.

The calves were housed in individual boxes, which were cleaned and disinfected daily with a commercially available quaternary ammonium product (Derdevice Plus Y, Deren Ilac, Turkey) against re-infection with environmental contamination. Strict biosecurity measures were implemented to prevent possible transmission and contamination between groups. The animals were fed with commercially available milk replacer (Eurolac Blue, Agrovit, Turkey) and had access to concentrate (Ilke, Abalim, Turkey) according to their body weight and age. Water and hay were provided ad libitum during the study period. No other medications were administered. After the completion of the study, the control calves were treated with chloroquine at the same dosage as the previously treated animals. The study protocol was approved by the institutional laboratory animals ethics committee of Adnan Menderes University HADYEK (with no: 2016/039 and date 25.02.2016). Prior to enrolment in this study, informed written consent was obtained from all of the owners/animal care takers.

Examination of fecal samples

The day of the first administration was determined as day 0. Collections from each calf were obtained on days 0 (before treatment), 3, 7 and 10 (after treatment). Fecal samples were collected manually from the rectum of all calves. Fecal material (1.5 g) was mixed through 33% $ZnSO_4$ solution (15 ml) and centrifuged at 880 x g for 5 minutes (Wilson et al., 2009). Fifty µl of fluid from the surface was emitted on a microscope slide containing Lugol iodine, which was covered by a slip. The slide was microscopically examined (400x power) for visualization of *Giardia* cysts. This procedure was repeated two times from different samples belonging to each calf by a single blinded researcher. The number of cysts per gram of feces (CPG) was calculated by [(number of cysts identified × 100)/1.5].

Assessment of efficacy of treatment

The efficacy of chloroquine treatment in the present study was assessed by microscopic examination of fecal samples collected on days 0, 3, 7 and 10 and measured based on the reduction in the number of CPG for the calves in the treatment group in comparison to the calves in the control group. The reduction in cyst excretion was calculated using the Henderson–Tilton formula, involving the geometric mean of the CPG similar to those of Geurden et al. (2010):

$$100\times \quad [\ 1-\frac{Ta \times Cb}{Tb \times Ca}\ ]$$

Ta and Tb represented the geometric mean CPG in the chloroquine treatment group before (Tb) and after (Ta) treatment with chloroquine, respectively; whereas Ca and Cb denoted the geometric mean CPG in the control animals before (Cb) and after (Ca) placebo treatment, respectively.

The Henderson-Tilton formula is considered as the most appropriate method as described and used previously by Geurden et al. (2010).

Molecular characterization of Giardia isolates

DNA was extracted directly from feces with QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's manual. Polymerase chain reaction (PCR)-based methods were employed to genotype using the procedures of Cacciò et al. (2002) and Lalle et al. (2005). Molecular characterization was carried out using PCR amplification and sequencing of the 511 bp region of the *β-giardin* gene.

Statistical analysis

The variables were tested for normality using the Shapiro-Wilk test. The group, time and group by time interactions were tested with repeated measures ANOVA. Related samples Friedman's two-way analysis of variance was done to control the statistical results of CPG before (day 0) and after the start of treatment (days 3, 7, 10) for each group. A Mann-Whitney-U test was used to compare differences between groups for each day. P values <0.05 were considered to indicate a significant difference. Software package (SPSS 22.0, SPSS Inc., Chicago, USA) was used for all tests.

RESULTS

Clinical sings

Calves in both groups presented clinical signs compatible with naturally occurring giardiosis, involving diarrhea on day 0. No other pathogens were detected in the feces of any of the calves during the study period. No observable and significant adverse reactions related with chloroquine treatment were noticed in the present study. All clinical signs including diarrhea had resolved on day 7 in all calves of the treatment group. Comparatively 5 out of 7 calves exhibited diarrhea on day 10, whereas diarrhea stopped in another 2 calves without treatment.

PCR amplification and sequence analysis of the β-*giardin* gene

Fecal samples of fourteen calves diagnosed *Giardia*-positive by the rapid test kits and microscopy, were positive by nested PCR (Figure 1). PCR products from each of the 14 *Giardia*-positive calf fecal samples were selected for molecular characterization. The β-giardin nested PCR assay was successfully applied to 14 isolates. The obtained DNA sequence from the selected isolates was compared with reference sequences (GenBank® accession number: M36728 for sub-assemblage A1, AY072723 for sub-assemblage A2 AY072724 for sub-assemblage A3). The sub-assemblage of all 14 isolates was determined as A3.

Cyst excretion

The geometric mean number of excreted cysts and reduction in cyst shedding are presented in Table 1. In the calves of the control group, cyst excretion on day 10 (ranging from 8000-140,000 CPG) was comparable to the initial excretion (ranging from 4000-340,000 CPG). Following chloroquine treatment, the reduction in cyst excretion calculated based on geometric mean was 99% on day 3 and 100% on days 7 and 10. The mean number of excreted cysts was significantly decreased ($p<0.001$) on days 7 and 10 after treatment.

DISCUSSION

G. duodenalis is an important intestinal pathogen of livestock. The number of studies on the prevalence

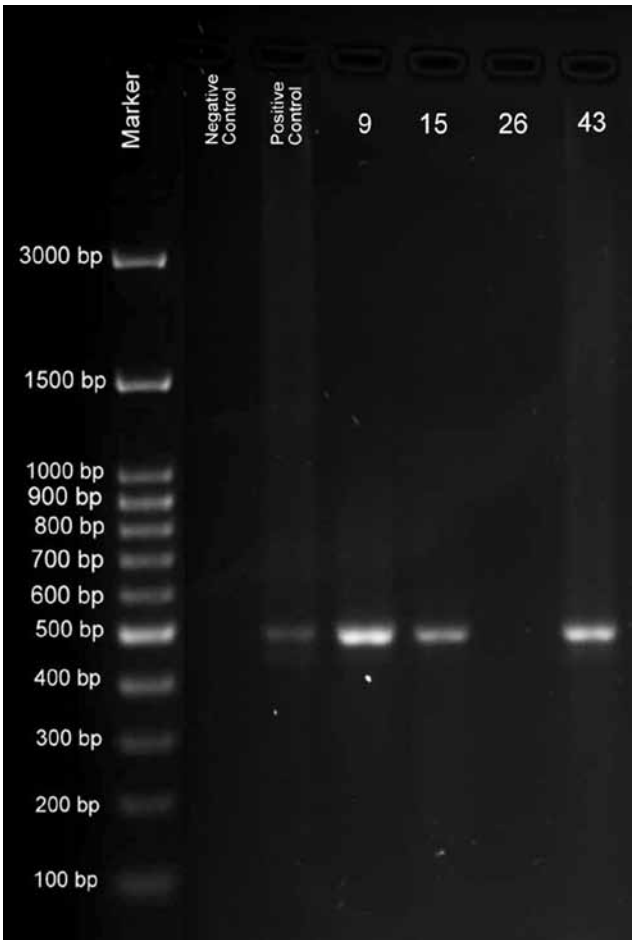

Figure 1. 2% Gel electrophoresis image of some of the positive samples in the nested PCR process (511 bp).

and molecular characterization of *G. duodenalis* in cattle in different parts of the world is increasing (Coklin et al., 2007; Gillhuber et al., 2013; Huang et al., 2014; Liu et al., 2015). However, only limited studies have been performed in Turkey on giardiosis in calves, with prevalence rates between 4.1% and 14.7% (Degerli et al., 2005; Goz et al., 2006; Gul et al., 2008).

Molecular characterization of *G. duodenalis* isolates in cattle has not yet been reported in Turkey. The vast majority of the previous studies detected assemblage E as the predominant genotype in livestock (O'Handley et al., 2000; Trout et al., 2007; Santín et al., 2009; Dixon et al., 2011), but shedding of assemblages A and B have also been reported in dairy cattle with mono or mixed infections (Cacciò et al., 2005; Geurden et al., 2008) and a recent study from different parts of the New York City watershed identified 100% of specimens from calves under 84 days of age, as assemblage A (Mark-Carew et al., 2012). Similarly, the present study revealed that all 14 of the *G. duodenalis* isolates collected from the calves, belonged to the sub-assemblage A3. Interestingly, the calves included in the study were from different farms in the Aydin region, but all samples belonged to same sub-assemblage. Assemblages of *G. duodenalis* may vary due to the geographical location and age as has been reported in previous studies (Winkworth et al., 2008).

Giardia assemblages A and B have been associated with their potential zoonotic role but recent molecular studies have demonstrated that the genetic structure of *Giardia* is more complex than thought before, and most of the sub-assemblages from animals do not share identical genotypes with humans (Sprong et al. 2009; Lebbad et al., 2010; Caccio, 2015). Epidemiologic studies have shown that most of the human isolates belong to sub-assemblages A2, whereas sub-assemblages A1 are found less often, and sub-assemblage A3 is not found at all (Sprong et al., 2009; Feng and Xiao, 2011). In this study, the sub-assemblage of *Giardia* isolates from the calves were described as A3 with the limited zoonotic risk. Only one gene (β-giardin) was targeted for genotyping the *G. duodenalis* positive samples, which is similar to what has been described elsewhere (Lee et al., 2016). Multilocus genotyping at the SSU rRNA, β-giardin, glutamate dehydrogenase (*gdh*), and triosephosphate isomerase (*tpi*) loci (Wang et al., 2014) was not performed.

Giardiosis should be treated with a practically applicable, safe, low-priced and highly effective protocol due to its high prevalence rates in calves, the risk of production losses by cause of clinical signs and the zoonotic potential, which is related with the genetic assemblage of the infection (Uehlinger et al., 2007). However, until this moment, relatively few options, specifically benzimidazole derivatives such as fenbendazole and albendazole, have been recommended for therapy against giardiosis in ruminants (Xiao et al., 1996; O'Handley et al., 2001; Geurden et al., 2010). With regard to the limited efficacy and therapy failure reports in human medicine (Argüello-García et al., 2009; Nabarro et al., 2015) and the lack of FDA approved drugs for the treatment of giardiosis in ruminants, there is clearly a need for alternative treatment options.

Chloroquine, an old but promising agent, has been re-identified as a possible treatment of giardiosis (Escobedo et al., 2015). This synthetic 4-aminoquinoline compound has been used as first-line treatment against malaria for many years (WHO, 2010). Currently, the compound is still widely used for uncomplicated malaria cases and is recommended as a second-line treatment option for several infectious and non-infectious diseases in humans (Escobedo et al., 2015).

The use of chloroquine in the treatment of giardiosis was first described by Basnuevo and Sotolongo, reporting the successful treatment of two human patients in 1946. Several case reports and studies with high success rates were published confirming the anti-giardial efficacy of chloroquine until 1965 (Lamadrid-Montemayor, 1954; Benetazzo and Tronca, 1955). After 1965, chloroquine was not used as a treatment choice for giardiosis anymore (Escobedo et al,. 2015). However, since 2000, two randomized clinical trials have been published in which chloroquine was given in a dose of 10 mg/kg twice daily for five days leading

Table 1. The geometric means of *G. duodenalis* cyst excretion in the control and chloroquine treated groups at each sampling day (before treatment [day 0] and after treatment [days 3, 7, 10]). The percentage of reduction calculated based on geometric means are presented.

	Day 0	Day 3	Day 7	Day 10
Control	27307.94[a]	17904.42[a]	15853.16[a]	32042.65[a]
Treatment	29777.96[a]	91.51[a,b]	0[b]	0[b]
p value	**0.902**	**0.128**	**0.001**	**0.001**
Reduction in cyst shedding		**%99**	**%100**	**%100**

*Different letters indicate significant differences between rows and columns ($p<0.05$).

up to 86% cure rate of giardiosis in children (Escobedo et al., 2003; Canete et al., 2010).

Antigiardial activity of chloroquine against *G. duodenalis* trophozoites has been demonstrated in in vitro studies (Gordts et al., 1985; Baveja et al., 1998; Nava-Zuazo et al., 2010). Although the mechanism of action of chloroquine is not completely understood, the efficacy of that compound is probably to be attributed to a reduced ability of the *Giardia* trophozoites to attach to the surface of the enterocytes (Baveja et al., 1998). Additionally, another hypothesis has been suggested that chloroquine might inhibit the function of the peripheral vacuoles in *Giardia* trophozoites (Tai et al., 1993).

Paramomycin, fenbendazole and albendazole have shown efficacy against giardiosis in calves (Xiao et al., 1996; O'Handley et al., 1997; Geurden et al., 2006). However, in a ninety-day study, it has also been reported that calves are mostly re-infected following fenbendazole treatment with *G. duodenalis* cysts in their environment (O'Handley et al., 2000). *Giardia* cysts may survive up to seven weeks in soil (Olson et al., 1999). Therefore, short treatment protocols might not be enough to prevent reinfection and should be combined with disinfection of the environment (Geurden et al., 2006). In the present study, the individual boxes of the calves were cleaned and disinfected every day of the study with a quaternary ammonium product. No reinfection occurred in the treatment group on days 7 and 10. However, based on other studies, it may be suggested that a long term follow-up is necessary in order to evaluate reinfection in calves (O'Handley et al., 2000; Geurden et al., 2006).

Chloroquine has only been studied in a few experiments in calves. Long term use of intramuscular chloroquine against *Onchocerca gutturosa* has shown promising results (Husna et al., 2010). Contrarily, a much earlier study revealed that it was ineffective against *Eimeria bareillyi* coccidiosis in buffaloes (Sanyal et al., 1985). However, no side effects were noted in both studies after parenteral chloroqine use in calves. Similarly, in the present study, no side effect was noticed related with oral chloroquine administration at a dose of 2.5 mg/kg, twice daily for five consecutive days.

To the authors' knowledge, the efficacy of an oral treatment with chloroquine against naturally occurring giardiosis in calves has been demonstrated for the first time. In the present clinical trial, chloroquine reduced the cyst excretion by 100% on days 7 and 10 after the start of the treatment without side effects. Additionally, chloroquine is an easily available and relatively cheap drug. In the present study, the cost per calf for a five-days therapy was 0.85 dollars (as was calculated by the total dose used) and thus would be a cost-effective alternative for the use against giardiosis in calves.

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The use of therapeutic soft contact bandage lenses in the dog and the cat: a series of 41 cases

Het gebruik van therapeutische zachte verbandcontactlenzen bij de hond en de kat: een reeks van 41 gevallen

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ABSTRACT

The objective of this case series was to illustrate in which cases soft contact bandage lenses can be used in the dog and the cat and how beneficial they are, supported by the literature. The benefit of the lenses was determined by how long the lenses stayed in place and if they contributed to the comfort of the patient. The average time the contact lenses stayed in place in both the dog and the cat was 9.8 days. Premature lens loss was seen in a limited number of cases (7% of the cases). Soft contact bandage lenses provided comfort and protection either in the initial healing phase, until the ulcer had healed (in cases of superficial corneal ulceration or cases of corneal ulceration with a limited stromal defect) or until surgery was performed (nasal trichiasis, entropion). In cases of corneal dystrophy, increased comfort for the patient was reported.

SAMENVATTING

De doelstelling van dit overzicht was om, ondersteund door de literatuur, te illustreren in welke gevallen een zachte verbandcontactlens gebruikt kan worden bij de hond en de kat en hoe nuttig ze zijn. Het voordeel van contactlenzen werd bepaald door na te gaan hoe lang de contactlens in het oog bleef en of ze het comfort van de patiënt verbeterden. De gemiddelde tijd dat de lens in het oog bleef, was 9,8 dagen (zowel bij de hond als de kat). Vroegtijdig verlies van de lens werd vastgesteld in een klein aantal gevallen (7%). De zachte verbandcontactlenzen verleenden comfort en bescherming ofwel in de aanvankelijke genezingsperiode, of tot het cornea ulcus (hoornvlieszweer) genezen was (in geval van een oppervlakkige zweer van het hoornvlies of een beperkt defect van het hoornvlies stroma) of tot op het moment dat chirurgie plaatsvond (voor trichiasis, entropion). In gevallen van corneale dystrofie werd een verbeterd comfort van de patiënt vastgesteld.

INTRODUCTION

Therapeutic soft contact bandage lenses have been used in general and referral small animal practice for some time. Already in 1977, Schmidt et al. found the use of hydrophilic contact lenses beneficial for superficial and recurrent corneal erosions in the dog and the cat. They have since been widely used in cases with indolent ulcers (superficial corneal erosions) to provide comfort and protection after a punctate or grid keratotomy for instance. An indolent ulcer or superficial corneal erosion is a superficial ulcer, which does not invade into the corneal stroma. These ulcers are typically associated with a loose non-adherent epithelial border. They are chronic in nature and are caused by a defect in the epithelial basement membrane and the superficial stroma (covered by an abnormal hyalinized acellular zone) thereby preventing normal re-epithelization of the corneal defect (Wooff et al., 2015). Soft contact bandage lenses can form an adjunct therapy in cases of corneal ulceration, to reduce pain and to retain the tear film over the ulcerated area, hence encouraging healing (Turner, 2008). In cases of corneal dystrophy, they provide comfort and pain relief. Corneal dystrophies in the dog are disorders of the cornea that are inherited, bilateral, non-inflammatory and not accompanied by systemic disease. Most cases appear as grey-white, crystalline or

Figure 1. Corneal dystrophy in a Shetland sheepdog.

Figure 2. Bausch & Lomb (PureVision, Balafilcon A) therapeutic soft contact bandage lens.

Figure 3. A therapeutic soft contact bandage lens prior to application.

metallic opacities in the central or paracentral cornea (Figure 1). Some cases which are non-inherited are sometimes named under the same umbrella; a more appropriate term in these cases is corneal degeneration. In some cases of corneal dystrophy and corneal degeneration, recurrent erosions develop, which are painful. Therapeutic soft contact bandage lenses are useful in these cases. They have been found useful in traumatic corneal injuries, such as corneal lacerations and perforations by providing comfort and protection. For cases of corneal edema, they can reduce discomfort and prevent epithelial bullae from rupturing. In cases of adnexal eyelid problems, such as distichiasis, trichiasis, entropion and eyelid neoplasia, they can provide comfort, protection and pain relief and furthermore prevent corneal abrasions from forming until surgery can be performed (Renwick, 2007; Turner 2008). In more complex cases, they can reduce trigeminal pain following entropion surgery in cases of pre-existing corneal ulceration, preventing associated blepharospasm (hence spastic entropion), and hereby prevent the need for repeated surgery (Turner, 2008). They have also been used as part of the surgical correction treatment for symblepharon in cats (Bedford, 1983) and dogs (Hendrix, 2013). This case series further illustrates in which cases contact lenses can be used, and gives valuable information on how successful the contact lenses used in this case series are by measuring how long they remained in the eye of the patient.

MATERIALS AND METHODS

The clinical notes were reviewed of cases (41 eyes) in which a therapeutic soft contact bandage lens was used. All cases were seen and reviewed by a veterinary ophthalmologist (the author of this paper) either in a primary or secondary (referral setting) at three veterinary centers (People's Dispensary for Sick Animals Pet Aid Hospital, 14 Newhall Road, S9 2QL, Sheffield, People's Dispensary for Sick Animals Pet Branch, Northern Avenue, S2 2EJ, Sheffield, United Kingdom and Highfield Veterinary Centre, 145-147 London Road, Sheffield, S2 4LH, United Kingdom) between January and September 2015.

All cases underwent a full ophthalmic examination prior to placement of the lens. Cases with abnormal purulent discharge, cases with tear-film abnormalities and cases with suspected bacterial keratitis were excluded. Contact lenses are not recommended in cases of dry eye (Schmidt et al., 1977).

In all cases, the therapeutic soft contact bandage lenses used were Bausch & Lomb Pure Vision lenses (Balafilcon A, 14 mm diameter) (Bausch and Lomb, 2016) (Figures 2 and 3).

Proxymetacaine was instilled in the eye on each occasion prior to lens application in order to aid in successful placement of the lens (Figure 4). Chloramphenicol drops were used as topical antibiotic eye

Figure 4. Placement of a soft contact bandage lens in a Dalmatian.

drops. Systemic non-steroidal inflammatory drugs were used in most cases as adjunctive therapy carprofen (Norbrook, United Kingdom) or meloxicam (Norbrook, United Kingdom) in the dog; meloxicam in the cat. In cases with significant reflex uveitis (in the dog), topical atropine 1% once daily in the affected eye was added. In selected cases in the dog (nasal fold resection, cat scratch, stromal ulcer in a Boxer), systemic antibiotics cephalexin (Shering-Plough, United Kingdom) or amoxicillin/clavulanic acid (Norbrook, United Kingdom) were added to the treatment regimen. One cat was on systemic clindamycin (Zoetis, United Kingdom) because of concurrent dental disease.

The age range of the patients was very varied and ranged from one year old to ten years and ten months old in the dog and from one year and ten months old to eighteen years and five months old in the cat.

RESULTS

Dogs

A total of 41 contact lenses were placed, i.e. 34 cases in dogs and 7 cases in cats (Tables 1 and 2).

In 59% of the cases, a contact lens was placed as an adjunct therapy for the treatment of a superficial corneal ulcer (corneal erosion) (Table 1). The routine treatment of a superficial corneal ulcer consisted of debridement followed by a punctate keratotomy mostly under local anesthesia (Figure 5). In three cases, the lens was placed under general anesthesia as an adjunct therapy: one case in a crossbreed had a concurrent cherry eye and the other two cases were particularly boisterous dogs (one Boxer and one Staffordshire bull terrier), which both had an initially debridement and punctate keratotomy, and for which a second procedure (debridement and punctate keratotomy) was required under general anesthesia, during which a new contact lens was placed. Contact lenses were placed at the end of the procedure to provide comfort and protection. In 29% of the cases, a contact lens was placed as adjunct therapy for a stromal ulcer (consisting of no more than a third to a half of the corneal stromal depth). In two of the cases, the stromal ulcer was caused by trichiasis and the contact lenses were placed prior to a nasal fold resection to provide comfort and protection. In one of these cases, a second contact lens was placed during surgery as adjunct therapy. In 6% of the cases, punctate superficial ulcers were treated with a contact lens as adjunct therapy. In 6 % of the cases, they were used for corneal dystrophy to provide comfort.

In 94% of the overall caseload in the dog, the contact lenses were effective in providing comfort and protection either in the early healing stage or until the ulcer was completely healed, and in providing comfort in corneal dystrophy cases without causing any adverse effects. Comfort was determined by markedly reduced or absent blepharospasm, the fact excessive lacrimation had ceased and whether the eye was open on review. The owner would also comment when questioned that the eye had opened up and had become comfortable (gradually reducing or absent blepharospasm).

In 6% of the overall caseload in the dog, the contact lens proved ineffective (premature loss of the contact lens) as the dog managed to remove the lens after a few hours on each occasion, even when wearing an Elizabethan collar.

In 24 dogs, it was possible to establish the (exact, confirmed on review appointment) average time frame of how long the lenses stayed in place. This specific contact lens (Bausch & Lomb PureVision) stayed in place for an average of 9.7 days in the cases, of which accurate information was available.

Figure 5. A superficial corneal ulcer in a Boxer after debridement.

Cats

In 57% of the cats, a contact lens was placed in cases of a superficial corneal ulcer (Table 2). The contact lens was used as adjunct therapy to provide comfort and protection. In 14% of the cases, it was used to provide temporary comfort prior to entropion surgery. In 14% of the cases, it was used as adjunct therapy for a stromal ulcer (consisting of no more than a third to a half of the corneal stromal depth).

In 86% of the overall caseload in the cat, the contact lenses were effective in providing comfort and protection either in the early healing stages or until the ulcer was completely healed, or in providing comfort (entropion) without causing any adverse effects. Comfort was determined by markedly reduced or absent blepharospasm, the fact excessive lacrimation had ceased and whether the eye was open on review. The owner would also comment when questioned if the eye had opened up and had become comfortable (gradually reducing or absent blepharospasm).

In 14% of the overall caseload in the cat, the contact lens proved ineffective (premature loss of the contact lens) as the cat managed the remove the lens after a few hours.

In two cats, it was possible to establish the (ex-

Table 1. Cases (34) in which a contact lens was placed in the dog.

Case number	Breed stayed in place	Problem information	Time lens	Result	Further
1	Lhaso apso	Superficial corneal ulcer	14 days (1)	Healed/confirmed	
2	Pug	(stromal) Corneal ulcer	?	Lost to Follow-up	
3	Boxer	Superficial corneal ulcer	3 weeks (1)	Healed/confirmed	
4	Shih tzu	(stromal) Corneal ulcer	2 weeks (1)	Healed/confirmed	Trichiasis case, had bilateral nasal fold resection
5	Crossbreed	(stromal) Corneal ulcer	4 days	Healing ++	Trichiasis case, had bilateral nasal fold resection
6	Crossbreed (*)	(stromal) Corneal ulcer	4 days	Healed/confirmed	Second lens placed (*) during nasal fold resection
7	Shih Tzu	(stromal) Corneal ulcer	?	Healed/confirmed	
8	Yorkshire terrier	Corneal dystrophy	> 5 days	Temporary comfort	Case of senile corneal dystrophy
9	SB terrier	Superficial corneal ulcer	7 days (1)	Healed/confirmed	
10	SB terrier	Superficial corneal ulcer	> 4 days	Healed/confirmed	
11	Boxer	(stromal) Corneal ulcer	7 days (1)	Healed/confirmed	
12	Crossbreed	(stromal) Corneal ulcer	?	Healed/confirmed	
13	Crossbreed	Superficial corneal ulcer	?	Healed/confirmed	
14	Boxer	Superficial corneal ulcer	?	Healing ++	Second lens placed (*)
15	Boxer (*)	Superficial corneal ulcer	?	Healed/confirmed	
16	Shih tzu	Punctate superficial ulcers	?	Healing ++	Second lens placed (*)
17	WHW terrier	Superficial corneal ulcer	18 days (1)	Healed/confirmed	
18	Boxer	Superficial corneal ulcer	?	Healed/confirmed	
19	SB terrier	Superficial corneal ulcer	?	Healing ++	Second lens placed (*)
20	Engl. Spr. spaniel	Superficial corneal ulcer	> 3 days	Lost to Follow-up	
21	Shih Tzu (*)	Punctate superficial ulcers	14 days (1)	Healed/confirmed	
22	SB Terrier (*)	Superficial corneal ulcer	?	Healed/confirmed	
23	Boxer	Superficial corneal ulcer	3 days	Healed/confirmed	
24	SB terrier	Superficial corneal ulcer	10 days (1)	Healed/confirmed	Distichiasis
25	Pug	(stromal) Corneal ulcer	half day	Ineffective (2)	This particular dog managed the remove the lens
26	Pug (*)	(stromal) Corneal ulcer	?	Ineffective (2)	even with a buster collar on, on 2 occasions (*)
27	Yorkshire terrier	Corneal dystrophy	22 days (1)	Healed/confirmed	corneal dystrophy with superficial corneal ulceration
28	Shih tzu	(stromal) Corneal ulcer	> 4 days	Healed/confirmed	Superficial cat-scratch
29	Crossbreed	Superficial corneal ulcer	27 days	Effective	Right eye, elderly dog, was euthanized for other health reasons
30	Crossbreed	Superficial corneal ulcer	27 days	Effective	Left eye, elderly dog, was euthanized for other health reasons
31	Greyhound	Superficial corneal ulcer	7 days (1)	Healed/confirmed	
32	Dalmatian	Superficial corneal ulcer	7 days (1)	Healed/confirmed	
33	Springer spaniel	Superficial corneal ulcer	7 days (1)	Healed/confirmed	
34	Sproker spaniel	Superficial corneal ulcer	6 days (1)	Healed/confirmed	

(1) Contact lens was removed and ulcer had healed
(2) Ineffective means the lens came out the same day and therefore was ineffective as adjunct therapy, the ulcer in case 25 and 26 was subsequently treated with a third eyelid flap and healed

Table 2. Cases (7) in which a contact lens was placed in the cat.

Case number	Breed stayed in place	Problem information	Time lens	Result	Further
1	Cat (DSH)	Entropion	?	Effective	Providing comfort
2	Cat (DSH)	Superficial corneal ulcer	11 days (1)	Effective	Provided comfort during healing process, FHV1
3	Cat (DSH)	(stromal) Corneal ulcer	?	Healed/confirmed	
4	Cat (DSH)	Superficial corneal ulcer	9 days (1)	Healed/confirmed	
5	Cat (DSH)	Superficial corneal ulcer	?	Healed/confirmed	
6	Cat (DSH)	Superficial corneal ulcer	?	Healed/confirmed	
7	Cat (DSH)	Superficial corneal ulcer	?	Ineffective (2)	Cat managed to remove lens, herpetic ulcer

DSH: Domestic shorthair
(1) Contact lens was removed and ulcer had re-epithelialized
(2) Ineffective means the lens came out the same day and therefore was ineffective as an adjunct therapy, the ulcer in this case eventually healed with time, without a contact lens

act, confirmed on review appointment) average time frame of how long the lenses stayed in place. This specific contact lens (Bausch & Lomb PureVision) stayed in place for an average of 10 days in the cases, of which accurate information was available, which is similar to the above figure in the dog.

In cats, it is generally more difficult to fit contact lenses as the eyelid aperture is smaller and tighter than in the dog. Furthermore, the third eyelid tends to protrude more easily when placing the lens. Hence, it becomes more difficult to place the lens (patience and the help of an assistant to hold the patient are recommended).

It should be noted that the specific lenses used in the cases of this review are very soft and provide a very good fit in most dogs and cats as they gradually settle in onto the corneal surface/curvature. The dia-meter is generally fine although in larger breeds, a touch larger diameter could be useful. In cats with a tight eyelid aperture and in kittens, they may prove too large and therefore are not useful in such cases. The author recommends reviewing each case on a weekly base initially. Although these specific contact lenses can comfortably stay in situ for three to four weeks in some cases, the author would advocate to remove them after that period of time or to change them for ongoing cases such as corneal dystrophy cases.

Proxymetacaine was instilled in the eye prior to lens removal in order to aid in the successful removal of the lens.

DISCUSSION

Reviewing the literature, the most common usage of soft contact bandage lenses is as adjunct therapy for the treatment of superficial corneal erosions. In this case series, they were used in 61% (25 overall) of the cases for the treatment of superficial corneal ulcers in the dog and the cat. In 11 cases (44% of the corneal erosion group), the lens was removed at a review appointment and it was confirmed the ulcer had healed. In these cases, the average healing time and the time the lens had stayed in situ was 10.6 days. In the other 14 cases (56% of the corneal erosion group), the lens had either come out after 3-4 days (3 cases) or no accurate data of how long the lens had stayed in place was available (9 cases), but all cases healed with improved comfort reported by the owner. In two cases, the dog had indolent ulcers in both eyes and other complex disease issues, and was euthanized for concurrent severe systemic disease. In that particular case, the ulcers were slow healing but the lenses stayed in place for 27 days providing comfort to both eyes. At initial presentation, the dog had bilateral large corneal erosions with significant associated blepharospasm, which completely settled down after treatment and placement of a contact lens.

Looking at this case series, the lenses provided comfort and protection in either the initial phase of the healing process or until fully healed in cases of superficial corneal erosions in both the dog and the cat. Grinninger et al. (2015) found that the healing time with the use of soft contact bandage lenses as adjunct therapy is significantly shorter (mean 14 +/- 0 days) than in cases without the use of contact lenses (mean 36 +/- 17 days) in dogs. Wooff and Norman (2015) reported a statically significant decrease in median healing time in cases, in which a bandage lens was used after a linear grid keratotomy in cases of superficial corneal erosions in Boxers. Both this case series and the reported literature illustrate the benefits of the use of a soft contact bandage lens as an adjunct therapy in the treatment of superficial corneal erosions.

Although in most cases, the contact lenses were placed under local anesthesia, two dogs in this case series required general anesthesia to perform a second punctate keratotomy for the treatment of a superficial corneal erosion at which a second contact lens was placed as adjunct therapy. If the superficial corneal erosion is specifically large and/or the dog is not the easiest to keep still, sedation or general anesthesia is recommended in order to perform a complete punctate keratotomy. Furthermore, in dogs that are aggressive or very boisterous contact lenses are not recommended. Although the use of an Elizabethan collar

might be prudent in many cases (Turner, 2008), it is case-dependant (in the present case series, the usage of a collar was discussed with the client/owner on each occasion; many cases were fine without one).

Martinez (2010) reported that the best results with contact lenses are obtained in cases of corneal dystrophies and spontaneous recurrent erosion syndrome. In two cases (5% overall) of the present case series, the lenses were used in corneal dystrophy cases to provide comfort, with good result. In one of the cases, the contact lens stayed in place for 22 days.

Soft contact lenses can be useful in cases of adnexal disease (Renwick, 2007; Turner 2008). In four cases (10% overall) of the present case series, adnexal problems, such as trichiasis and entropion, were the reason for the use a contact lens either before surgery to provide protection or after surgery as continued treatment for a healing stromal ulcer.

The remainder of cases consisted of punctate superficial ulcers (two cases), a superficial cat-scratch (one case) and four cases of a stromal corneal ulcer (with a depth not exceeding more than a third to a half of stromal depth) demonstrating the diversity of cases, in which a soft contact bandage lens can be useful. All contact lenses stayed in place in these cases in the initial healing phase or until the defect was healed.

In some animals (7% of cases overall), the lenses came out prematurely. In this case series, one owner reported that his pet actively tried to remove the lens even with the wear of an Elizabethan collar. Premature loss of the lens has been reported in the literature (Morgan, 1984), but with the improvement in contact lens technology, newer lenses have a much better fit. The lenses used in this case series are specifically soft and settle into the eye nicely. These particular lenses start to take on the shape of the cornea after only a few minutes or less. Concerns regarding air bubbles under the lens, which are seen with some commercially available veterinary bandage lenses in the UK (Turner, 2008), are much less of a problem as they tend to dissolve after a few minutes.

CONCLUSION

Soft contact bandage lenses, in specific Bausch & Lomb Pure Vision (Balafilcon A), are useful as an adjunct therapy in several conditions reaching from superficial corneal ulcers, corneal dystrophies, adnexal disease to superficial stromal defects. The average time the lenses stayed in situ in this case series (in both dogs and cats) was 9.8 days with a low rate of premature lens loss (7%). They are particularly useful in superficial corneal ulcers, which is also confirmed in this case-series (Gosling et al., 2013; Grinninger et al., 2015; Wooff et al., 2015).

It is highly recommended to perform a thorough ophthalmic examination in each case. Soft contact bandage lenses are just one of the tools in the ophthalmic toolbox, they are not a quick fix and cases have to be assessed for suitability.

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Congenital primary hypothyroidism in a cat

Congenitale primaire hypothyroïdie bij een kat

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ABSTRACT

A five and a half-month-old, male domestic shorthair of 1.4 kg was presented with severe constipation. Physical examination showed a dull, small cat with a poor hair coat and excessive scaling, hypothermia and a large amount of feces in the abdomen. Body proportions showed disproportional dwarfism with a large head and a short neck and limbs. Radiographs revealed marked epiphyseal dysgenesis with delayed maturation and ossification. Megacolon was present. Based on an undetectable level of TT_4 and an elevated TSH level in serum, congenital primary hypothyroidism was diagnosed. On scintigraphic examination, the diagnosis was confirmed. After several months of levothyroxine therapy, the cat was bright and alert, showed no signs of constipation and developed normally.

SAMENVATTING

Een vijf en een half maanden oude, mannelijke, intacte Europese korthaar van 1,4 kg werd aangeboden met de klacht van erge constipatie. Op het lichamelijk onderzoek viel op dat de kat erg klein en suf was, een slechte vachtkwaliteit met veel schilfers vertoonde, hypothermie en veel ontlasting in het abdomen had. De lichaamsverhoudingen waren duidelijk uit proportie waarbij een groot hoofd, een korte nek en korte ledematen opvallend waren. Op het radiografisch onderzoek waren een vertraagde sluiting van de groeiplaten zichtbaar en een duidelijk megacolon. Gebaseerd op een onmeetbaar laag totaal T4 en een verhoogd serum TSH werd congenitale primaire hypothyroïdie gediagnosticeerd. Het scintigrafisch onderzoek bevestigde deze diagnose. Verscheidene maanden na het opstarten van een levothyroxinetherapie was de kat actief en alert. Hij vertoonde geen tekenen meer van constipatie en ontwikkelde zich verder normaal.

INTRODUCTION

Congenital primary hypothyroidism is a rare endocrine condition in cats. Only a small number of papers describing different etiologies of congenital or spontaneous adult-onset hypothyroidism in cats have been published (Crowe, 2004; Mellanby et al., 2005; Traas et al., 2008; Quante et al., 2010; Galgano et al., 2014; Lim et al., 2014). The congenital form is more common than the naturally acquired form, although both are extremely rare (Bojanic et al., 2011). The incidence of congenital hypothyroidism is unknown because a subset of cases is not diagnosed. Congenital primary hypothyroidism causes disproportionate dwarfism, which leads to kittens having a large head and short neck and limbs. (Crowe, 2004; Scott-Moncrieff, 2007). Other common clinical signs are lethargy, mental dullness, delayed dental eruption, constipation, bradycardia and hypothermia (Scott-Moncrieff, 2007; Nelson, 2009; Bojanic et al., 2011; Daminet, 2012).

In this case report, the diagnosis, treatment and outcome of a kitten with congenital primary hypothyroidism are described, and its purpose is to make clinicians aware of this condition. It rarely occurs but any clinician should try to recognize this condition as the prognosis improves with fast installment of the treatment.

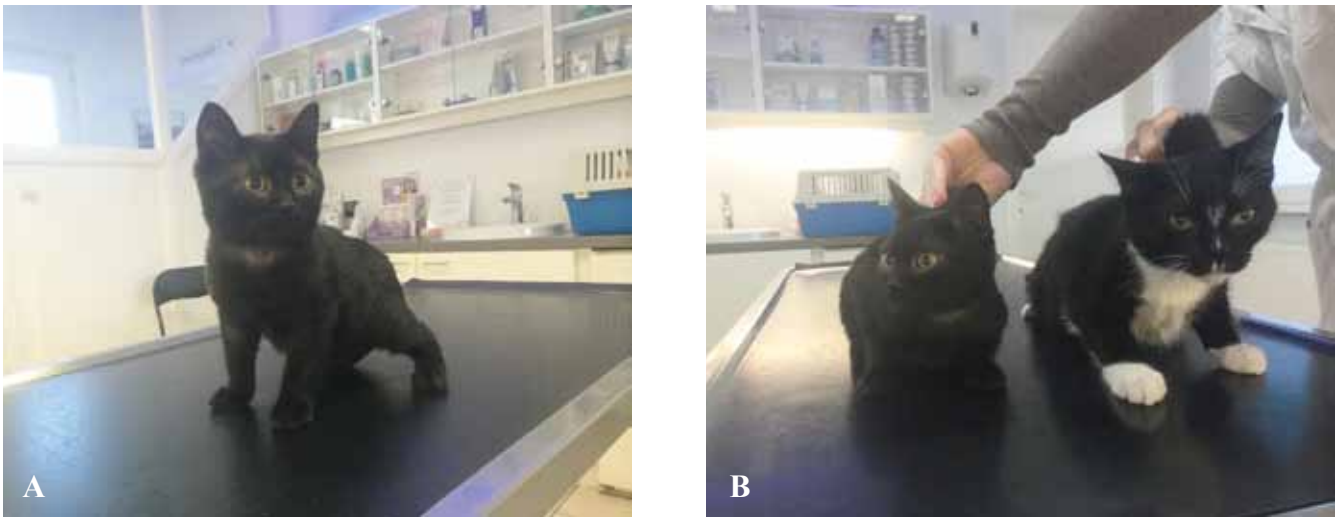

Figure 1. A. five-and-a-half-month-old, intact, male domestic shorthair. Note the disproportion of his body with a large head and short limbs. B. The hypothyroid cat next to a comparably aged cat to show his small size.

CASE REPORT

A five-and-a-half-month-old, intact, male domestic shorthair kitten was presented with a two-day history of constipation. The kitten was found in the backyard of the owner, together with his littermates. The cat was adopted by the owner when he was about two months old. The kitten was clearly smaller than his littermates. He had always been very calm and slept more than the other cats in the household. Since there were no other complaints, the owner did not pay any further attention to it. At the moment of presentation, the owner informed that the kitten had had problems making stool since two days. Examination revealed a dull, small cat with a body weight of 1.4kg. The body temperature was 37.2°C. Furthermore, the cat had a poor hair coat with excessive scaling and all deciduous teeth were still present. His body was disproportionate, with a large head, short neck and short limbs (Figures 1A and 1B). The thyroid gland was clearly palpable. The colon was clearly distended on palpation due to severe constipation. Radiographic examination showed epiphyseal dysgenesis and mildly widened vertebral physes (Figures 2A and 2B). Megacolon was also present containing a large amount of feces. The feces were successfully removed manually under anesthesia and a blood sample was taken. To ameliorate the constipation and suspected colitis, oral lactulose and metronidazole (10 mg/kg q24h) were initiated.

The two main features in this cat were disproportionate dwarfism and megacolon. The differential diagnoses for dwarfism are congenital hypothyroidism hyposomatotropism, chondro-dystrophy, poor quality diet or inadequate caloric intake, gastro-intestinal disorders or parasitism, congenital cardiac anomaly, juvenile diabetes mellitus, portosystemic shunt, hypoadrenocorticism, renal disorder or lysosomal storage diseases (Nelson, 2009; Lim et. al., 2014). Poor quality diet or inadequate caloric intake was less likely, because the cat had a good appetite and was eating

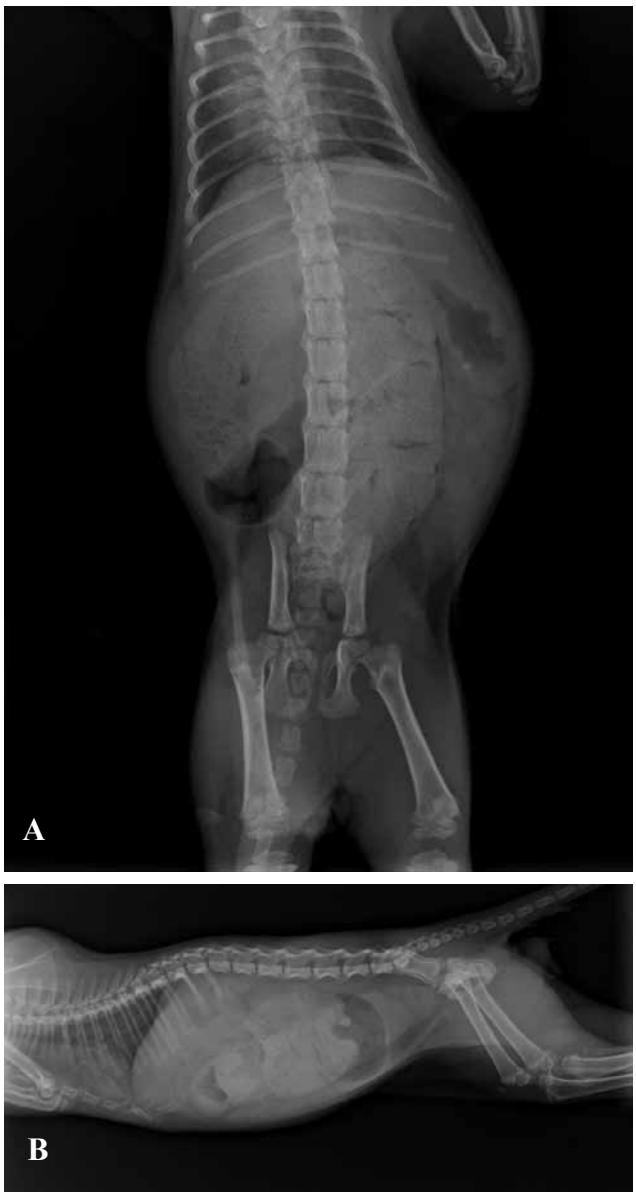

Figure 2. A. Ventrodorsal and B. right lateral radiographs showing delayed ossification and widened growth plates. Note the widened and square vertebral bodies. Megacolon is also present.

a well-balanced commercial diet. No indications for gastro-intestinal or cardiac disease were present in the history or on physical examination, making gastro-intestinal disorders, parasitism or a congenital cardiac anomaly unlikely.

Hematology and serum biochemistry revealed a slightly lowered hematocrit (20.4%; reference rate (RR) 24.8 – 37.5%), erythrocyte count (4.52 x 10^12/l; RR 5.43 – 10.22 x 10^12/l) and hemoglobin (7.1 g/dl; RR 8.0 – 12.9 g/dl), a mild leukocytosis (22.44 x 10^9/l; RR 5.50 – 19.50 x 10^9/l) due to an increase in mature neutrophils (17.48 x 10^9/l; RR 2.50 – 12.50 x 10^9/l) and increased alanine aminotransferase (152 U/l; RR 12 – 115 U/l). Together with a reticulocyte count and reticulocyte index within normal limits, the mild anemia was considered to be non-regenerative. The results of the blood analysis excluded renal disorders and juvenile diabetes mellitus as causes of the dwarfism. Electrolytes (sodium, potassium, calcium and phosphorus) and cortisol were within reference ranges, so hypoadrenocorticism was very unlikely. Vitamin B12 turned out to be normal and this result excluded dwarfism as a result of decreased serum cobalamin levels. The total serum thyroxine (TT_4) level was undetectable (< 0.5 µg/dl; RR 1.1 – 3.5 µg/dl). Marked elevation of the thyroid stimulating hormone (TSH) level was detected, using a canine-specific chemiluminescent immunoassay (ECLIA) (6.70 ng/ml; RR 0 – 0.6 ng/ml). Insulin-like growth factor 1 (IGF-1) was measured to investigate for hyposomatotropism and was found to be normal (450.3 ng/ml; RR 48.4 – 544.0 ng/ml). Liver function was further investigated by measuring blood ammonia concentration and pre- and postprandial bile acids, but since they were within normal limits, a portosystemic shunt could be excluded. Based on the low serum TT4, the elevated cTSH and the normal IGF-1, primary hypothyroidism was diagnosed in this cat.

To confirm the diagnosis of congenital primary hypothyroidism, the cat was referred for a diagnostic thyroid scintigraphic scan. For this purpose, 117 MBq of sodium pertechnetate ($Na^{99m}TcO_4$) was injected intravenously through an indwelling catheter in the cephalic vein. A planar static scan was performed 20 minutes after administration of the radiofarmaceutical, with the cat in sternal position above the gamma-camera. Both thyroid glands were severely enlarged and demonstrated an increased uptake of $Na^{99m}TcO_4$ (Figures 3A and 3B). Thyroid function is often expressed as the ratio of uptake in the thyroid gland in comparison to the salivary gland uptake (normal ratio is approximately 1/1). However, in this patient, the salivary glands could not be reliably delineated. Alternatively, the percentage of the injected dose of $Na^{99m}TcO_4$ that is accumulated in the thyroid gland can be calculated (%TcU), although there is a wide range of normal values reported in the literature, from 0.25 to 3.9% (Mooney et al., 1992; Nap et al., 1994; Daniel et al., 2002; Daniel and Brawner, 2006; Lee et al., 2010). The patient's uptake was markedly increased with 7.38 % TcU in the left and 6.15 % TcU in the right thyroid gland.

Treatment with levothyroxine was started at an initial dose of 50µg once daily. After four weeks, blood analysis was repeated: the non-regenerative anemia and leukocytosis had been normalized, and the previously reported increased alanine aminotransferase was within normal ranges. Total T4 had increased to the low normal range but TSH was still too high (1.2 ng/ml), despite its marked decrease. Therefore, the levothyroxine dosage was increased to 50µg twice a day. The cat was reevaluated several times during the following months and two more adjustments of the

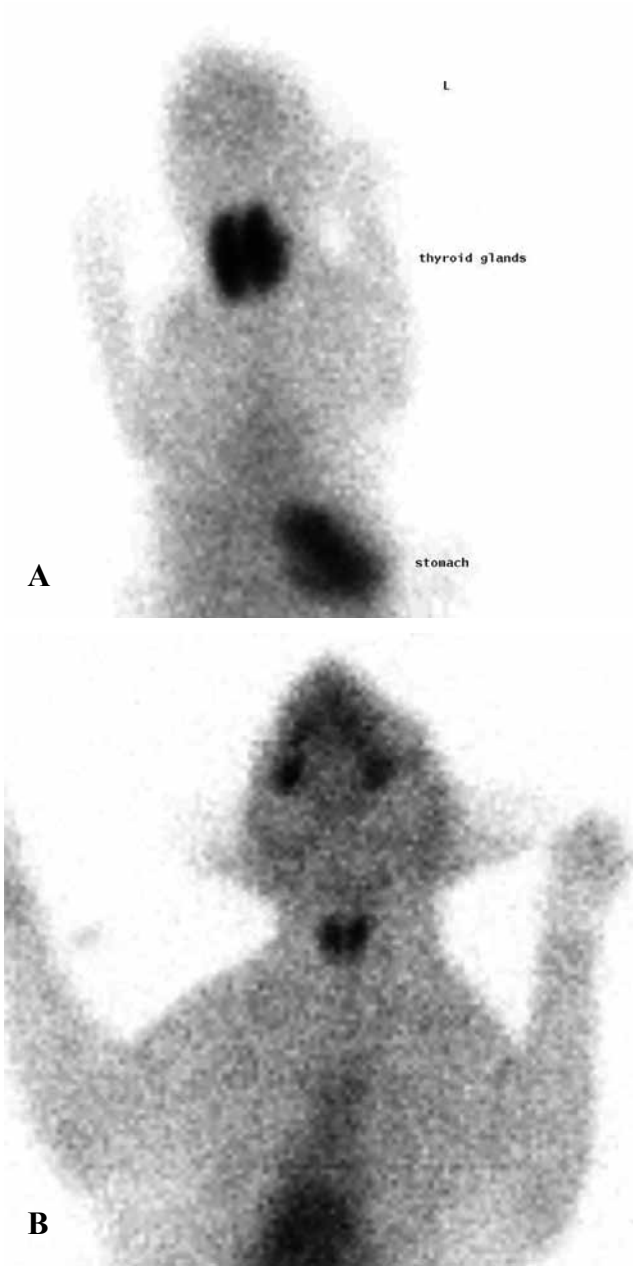

Figure 3. A. Ventral static acquisition of the patient. Both thyroid glands are markedly enlarged and have an increased pertechnetate uptake. Pertechnetate activity in the stomach is physiologic. Salivary glands are non-discernable in this patient. B. Similar scan of a cat with normal thyroid function.

levothyroxine dosage were implanted. Finally, the cat was doing clinically well with normal hematologic and biochemical parameters on a dose of 100µg twice a day. Three months after the diagnosis, at the age of nine months, radiographs were repeated and showed complete closure of the growth plates (Figures 4A and 4B). The cat had gained weight (up to 3.1 kg) and the permanent teeth were present. No complaints of constipation had been noticed by the owner and the therapy with lactulose was stopped. At the age of one year, the cat was still doing well at the same levothyroxine dose and had the appearance of a perfectly normally developed cat (Figure 5).

Figure 4. A ventrodorsal and B right lateral radiographs showing complete closure of the growth plates. No signs of megacolon were present.

DISCUSSION

Most cases of hypothyroidism in cats consist of iatrogenic hypothyroidism after receiving radioactive iodine (^{131}I) or oral methimazole or thiamazole as a treatment for hyperthyroidism (Nykamp et al., 2005). HHH ypothyroidism can be classified as primary (due to thyroid disease), secondary (inadequate secretion of thyroid stimulating hormone) or tertiary (inadequate secretion of thyroid releasing hormone) (Traas et al., 2008; Lim et al., 2014). In cats, most cases describing hypothyroidism suffer from primary disorders of the thyroid gland and mainly kittens are affected (congenital form) (Jones et al., 1992; Crowe, 2004; Traas et al., 2008; Quante et al., 2010; Galgano et al., 2014; Lim et al., 2014). Spontaneous adult-onset primary hypothyroidism is far more uncommon and only three well-documented cases have been reported (Rand et al., 1993; Blois et al., 2010; Galgano et al., 2014). One case of a cat developing hypothyroidism following head trauma has been reported (Mellanby et al., 2005). Even though congenital hypothyroidism is a rare condition in cats, the actual prevalence may be higher than reported as probably many kittens may have died undiagnosed or misdiagnosed as idiopathic megacolon (Traas et al., 2008; Lim et al., 2014). Because thyroid hormones are essential for normal postnatal development, hallmarks of congenital hypothyroidism are disproportionate dwarfism and delayed epiphyseal ossification (Greco, 2005). Disproportionate dwarfism is characterized by a large and broad skull, a short neck and short limbs and a wide trunk (Bojanic et al., 2011). Other clinical signs of congenital hypothyroidism that could be recognized are mental dullness, lethargy, constipation, dry skin and excessive scaling, delayed dental eruption, bradycardia and hypothermia (Nelson, 2009; Daminet, 2012). These signs are usually not present at birth, but develop postnatally and will become obvious to owners by the age of eight to twelve weeks, which

Figure 5. The same cat as in Figures 1A and 1B at the age of one year. He has the appearance of a normally developed cat.

was also the case in this cat (Bojanic et al., 2011). When congenital hypothyroidism is suspected based on clinical signs, a diagnosis can be made by measuring total T4 and endogenous TSH concentrations. Affected cats are expected to have low total T4 and high TSH concentrations if the cause is thyroid-dependent. Since there is no feline-specific TSH assay available, a chemiluminescent immunoassay for canine TSH is used to measure feline TSH concentrations (Greco, 2006; Galgano et al., 2014).

Primary congenital hypothyroidism can be divided into two main categories: thyroid dyshormonogenesis (goitrous) and dysmorphogenesis (non-goitrous). The goitre in the thyroid dyshormonogensis category is the result of an increased TSH concentration in response to low thyroid hormone concentrations and subsequent thyroid hyperplasia. In case of thyroid dysmorphogenesis, there are defects to the TSH receptor, which lead to development defects and aplasia of the thyroid gland (Quante et al., 2010; Bojanic et al., 2011). In the cat of the present case, the thyroid glands were palpable and scintigraphy showed an increased uptake of $Na^{99m}TcO_4$, so thyroid dyshormonogenesis was suspected. Increased uptake of $Na^{99m}TcO_4$ indicates a functional NaI-symporter transport mechanism (Quante, 2010). Thyroid dysmorphogenesis is less likely as aplastic thyroid glands would not be visible on these scans. $Na^{99m}TcO_4$ is routinely used in thyroid scintigraphy, as it mimics the biologic behavior of iodine to a certain extent. The uptake mechanism of both $Na^{99m}TcO_4$ and iodine into the thyrocytes uses the NaI-symporters. However, $Na^{99m}TcO_4$ will not be incorporated into thyroid hormones (no further organification). ^{123}I on the other hand undergoes organification and would have been the best tool to describe the mechanism of congenital hypothyroidism in this case. Despite the less accurate reflection of the thyroid function compared to ^{123}I, $Na^{99m}TcO_4$ is considered appropriate to evaluate thyroid function. Further, the cost of radioactive iodine isotopes (^{123}I and ^{131}I) are higher than the readily available $Na^{99m}TcO_4$. Lastly, even though ^{131}I has reportedly been used in low doses, it holds a radiotoxic component (beta-particle decay) that is useful for therapeutic purposes but also contributes to a higher radiation burden for the patient.

Defects during the synthesis of thyroid hormones may occur at several levels, such as impaired uptake of iodine by the thyroid gland (through the NaI-symporter) or deficient organification (by thyroid peroxidase and thyroid oxidase-2 enzymes) and transport (by pendrin) of iodine (Bojanic et al., 2011). Jones et al. (1992) reported an organification defect in a family of Abyssian cats with an autosomal recessive mode of inheritance. IV administration of sodium perchlorate as an active competitor for $Na^{99m}TcO_4$ (or radioactive iodine isotopes) in the thyroid glands is known as a technique to pinpoint the pathology more exactly, but was not pursued in this case.

The diagnosis of hypothyroidism was made and levothyroxine therapy was started. Several months and a few dosage adjustments later, signs of hypothyroidism resolved and the cat was doing well. Cats that suffer from congenital hypothyroidism and receive levothyroxine therapy for this condition may have a good prognosis, as in the present case. The long-term outcome however is unknown, but depends on the etiology and the age when treatment is initiated, since thyroid hormone is necessary for the normal development of bones, joints and the central nervous system (Bojanic et al., 2011).

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Combined atypical primary hypoadrenocorticism and primary hypothyroidism in a dog

Gecombineerde primaire atypische hypoadrenocorticisme en primaire hypothyroïdie bij een hond

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ABSTRACT

A dog with combined atypical primary hypoadrenocorticism and primary hypothyroidism is described. The dog presented with waxing and waning, vague complaints since more than a year and had been treated with several drugs without complete resolution of signs. Based on the abnormalities on physical examination, blood examination and abdominal ultrasonography, atypical primary hypoadrenocorticism and primary hypothyroidism were diagnosed. Glucocorticoid supplementation was started and gradually tapered to maintenance rate because of polydipsia. Ten days later, levothyroxine supplementation was started at a very low dose and was gradually increased based on serum total thyroxine concentrations. The dog rapidly improved and recovered completely. Follow-up over a one-year period did not reveal new abnormalities. The presence of combined primary hypoadrenocorticism and primary hypothyroidism has been infrequently described in dogs and may resemble the Schmidt's syndrome in humans.

SAMENVATTING

In deze casus wordt een hond met gecombineerde atypische primaire hypoadrenocorticisme en primaire hypothyroïdie beschreven. De hond vertoonde reeds meer dan een jaar intermitterende, vage klachten. Ondanks meerdere medicamenteuze behandelingen vertoonde de hond geen volledige beterschap. Gebaseerd op de resultaten van het algemeen lichamelijk onderzoek, het bloedonderzoek en de abdominale echografie werd de hond gediagnosticeerd met primaire atypische hypoadrenocorticisme en primaire hypothyroïdie. Een supplementatie met glucocorticoïden werd meteen opgestart en geleidelijk afgebouwd tot een onderhoudsdosis wegens polydipsie. Levothyroxinesupplementatie werd tien dagen nadien opgestart aan een zeer lage dosis en geleidelijk opgebouwd aan de hand van de totale thyroxine-serumconcentratie. De hond verbeterde snel en herstelde compleet. Controleconsultaties gedurende meer dan een jaar toonden geen nieuwe abnormaliteiten aan. De combinatie van primaire hypoadrenocorticisme en primaire hypothyroïdie wordt niet vaak beschreven bij honden en vertoont gelijkenissen met het humane schmidtsyndroom.

INTRODUCTION

Addison's disease, also called primary hypoadrenocorticism (HA), is a well-known, yet uncommon endocrinopathy, in which the adrenal glands are incapable of producing sufficient concentrations of steroids (glucocorticoids and/or mineralocorticoids) (Scott-Moncrieff, 2010; Baumstark et al., 2014). Female, young to middle aged dogs have a predisposition for this disease (Sadek and Schaer, 1996; Thompson et al., 2007). The steroid deficiency can be due to destruction of the adrenal cortex (primary HA), mostly caused by an immune mediated process, or due to pituitary dysfunction (secondary HA) (Kintzer and Peterson, 1997; Scott-Moncrieff, 2010). In a minority of cases with primary HA, only the zona fasciculata and zona reticularis and not the zona glomerulosa of the adrenal cortex are affected (Sadek and Schaer, 1996). Thereby, only the glucocorticoid production is impaired and no electrolyte abnormities are noted. This is referred to as atypical primary HA (Sadek and Schaer, 1996; Lifton et al. 1996,). Sodium

and potassium concentrations may also be normal in dogs with secondary HA due to adrenocorticotropic hormone (ACTH) deficiency as this deficiency does not impair mineralocorticoid production (Thompson et al., 2007). Atypical primary HA often causes vague complaints with a waxing and waning history, such as anorexia, vomiting, lethargy, depression, weakness, weight loss, diarrhea and shaking or shivering (Melendez et al., 1996; Kintzer and Peterson, 1997; Thompson et al., 2007; Scott-Moncrief, 2010). On physical examination, non-specific abnormalities can be noticed or the physical examination can even be unremarkable (Thompson et al., 2007; Scott-Moncrief, 2015). Hematology and serum biochemistry results show only subtle clinicopathological changes, such as absence of a stress leukogram, mild non-regenerative normocytic, normochromic anemia and hypoglycemia (Scott-Moncrief, 2010).

Dogs with clinical signs as described above, which do not react properly to symptomatic treatment, and do not show a stress leukogram, may be suspected of atypical HA and further testing is recommended. The gold standard to confirm the diagnosis of HA is an ACTH-stimulation test. Serum cortisol should be measured prior to and 60 to 90 minutes after ACTH administration (Scott-Moncrieff, 2010; Scott-Moncrieff, 2015a). To differentiate between primary and secondary HA, it is necessary to evaluate the pituitary function by evaluating ACTH-stimulated aldosterone concentrations or basal canine ACTH concentrations (Scott-Moncrieff, 2015a).

In primary HA, the destruction of the adrenal gland is mostly caused by an immune-mediated process which, in rare cases, can occur concurrently with other immune-mediated endocrine disorders, such as hypothyroidism (HT), diabetes mellitus and hypoparathyroidism (Melendez et al., 1996; Scott-Moncrieff, 2015a: Scott-Moncrieff, 2015b). In one study, 5 % of the 225 dogs with HA had concomitant endocrinopathies (Peterson, 1996). The presence of multiple endocrine disorders in dogs resembles polyglandular deficiency syndrome in humans (Neufeld et al., 1980).

In the present case, a dog is presented that was diagnosed with a combination of atypical primary HA and primary HT compatible with a type II polyglandular syndrome, or the Schmidt's syndrome in humans. Both atypical HA and Schmidt's syndrome are rare and poorly characterized endocrinopathies in the dog. The purpose of this case report is to make this disease entity more familiar to practicing veterinarians.

CASE REPORT

A six-year-old, female, neutered, mixed-breed dog of 26 kg was presented at the Faculty of Veterinary Medicine of Ghent University. The complaints had started more than a year before admission. Summarized, the dog had a waxing and waning history of exercise intolerance, depression, drowsiness, partial anorexia, nausea, vomiting, ptyalism, diarrhea, polydipsia, sneezing, nasal discharge, purulent ocular discharge, conjunctivitis, otitis externa due to malassezia infection, hair loss and reduced skin and coat quality. Several physical and blood examinations had been performed by different veterinarians. Additionally, urinalysis, serological tests, abdominal ultrasonography and echocardiography had been performed. According to the referring veterinarians, the physical examination did not reveal significant abnormalities, except for mild generalized lymphadenopathy two months prior to admission. Serum biochemistry profiles revealed moderate azotemia, mild polyclonal hypergammaglobulinemia, very mild hyperkalemia, mild hypoglycemia, increased lactate dehydrogenase (LDH) and moderately increased creatine kinase (CK) (Table 1). On complete blood count (CBC), a mild microcytic, hypochromic anemia and lymphocytosis were noticed (Table 1). Additional blood tests revealed increased total thyroxine (TT4), increased Borrelia IgG and a negative Leishmania screening (direct agglutination test). Because of mild hypoglycemia, the insulin concentration was also evaluated to rule out insulinoma and this was below reference interval (6.3 mU/L (9.0-25.0)). The higher described abnormalities on serum biochemistry and CBC were not all consistently present. Complete urinalysis of free catch urine revealed isosthenuria (USG 1.014), rare calcium oxalate crystals and a negative culture. On abdominal ultrasonography, two months before admission, the kidneys were mildly decreased in size (5.4-5.9 cm), but showed no other abnormalities. The adrenal glands had a normal shape, but were also small (3.9-5.2 mm). There was a small amount of sediment in the urinary bladder and the medial iliac lymph nodes were prominent, but had a normal echogenicity. The peripheral lymph nodes were also ultrasonographically evaluated at that time and appeared mildly heterogeneous and more oval shaped. Cytology of the peripheral lymph nodes was suggestive for reactive lymphadenopathy. For all previously summarized vague complaints, several symptomatic treatments were attempted (metoclopramide, nandrolonlaurinaat, vitamin B complex, ranitidine, metronidazole, cyclosporine, enrofloxacine, meloxicam, doxycycline, probiotics, Surolan® (miconazolenitrate, polymyxine-B-sulfate, prednisoloneacetate), Aurizon® (marbofloxazine, clotrimazole, dexamethasone), maropitant, omeprazole). Most of these therapies had no effect or only yielded temporary, partial amelioration. Because of the positive Borrelia serology, doxycycline was given for a period of four weeks. However, as none of the treatments resulted in complete recovery and because of ongoing exercise intolerance, weakness and stiff gait, the dog was referred for a suspicion of a diffuse peripheral neuromuscular problem.

On physical examination at the Department of Medicine and Clinical Biology of Small Animals, Faculty of Veterinary Medicine of Ghent University,

Table 1. Complete blood count, serum biochemistry profile, electrolyte concentrations, hormonology from the referring veterinarians expressed in '# weeks before admission'.

Variable		Units	45 weeks	8 weeks	7 weeks	6 weeks	4 weeks	Reference interval
CBC								
	HCT	%	41.4	/	35.8	/	36.2	43.0-59.0
	Hemoglobine	mmol/L	8.66	/	7.60	/	/	8.72-12.46
	MCV	fl	63.2	/	60.4	/	/	63.0-77.0
	MCH	pg	21.2	/	20.6	/	/	21.0-25.0
	WBC	/µL	10610	/	12190	/	/	6000-16000
	Segmented	/µL					/	3000-11500
	neutrophils		4371	/	4888	/		
	Band neutrophils		0	/	0	/	/	0-300
	Lymphocytes	/µL	5379	/	6522	/	/	1000-4800
	Monocytes	/µL	329	/	280	/	/	<1350
	Basophils	/µL	11	/	24	/	/	0-100
	Eosinophils	/µL	520	/	488	/	/	<1250
	Thrombocytes	K/µL	274000	/	312000	/	/	164000-510000
	Reticulocyte	%	0.8	/	0.3	/	/	
Biochemistry								
	Urea	mmol/L	13.49	7.83	5.16	8.82	8.16	0.99-9.49
	Creatinine	µmol/L	98.1	256.4		160.9	136.1	<60
	Total protein	g/L	56	58	61	69	69	53-80
	Albumin	g/L	/	22.0	24.1	29	32	22-44
	Gamma globulines	g/L	/	11.3	11.7	12.3	10.4	3.6-9.1
	Glucose	mmol/L	3.39	3.55	3.61	/	/	3.05-4.99
	ALT	U/L	65	/	122	/	/	<70
	AST	U/L	69	/	69	/	/	<50
	ALP	U/L	33	/	41	/	/	<111
	GGT	U/L	5	/	7	/	/	<9
	LDH	U/L	/	/	676	/	584	<605
	Bile acids	U/L	/	/	8	/		<19
	CK	U/L	/	/	1359	/	319	<331
Electrolytes								
	Sodium	mmol/L	/	/	145	/	/	143-154
	Potassium	mmol/L	/	/	5.9	/	/	4.2-5.6
	Calcium	mmol/L	/	/	2.54	/	2.64	2.02-2.85
	Phosphorus	mmol/L	/	/	1.08	/	1.87	0.96-1.88
Hormonology								
	TT4	nmol/L	60.63	/	/	/	/	6.45-43.86

HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, WBC: white blood cells, ALT: alanine transferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: Gamma globulinetranferase, LDH: lactate dehydrogenase, CK: creatinine kinase, TT4: total thyroxine. Increased values are colored in red, decreased values are colored in green.

the dog appeared slow, dull and depressive (Figure 1). Inspection of the dog revealed a stiff gait on the fore limbs and lethargy. A video of a walk with the owner also showed exercise intolerance and weakness. The heart rate (80 beats per minute), the respiration rate (20 per minute) and the temperature were all normal. Cardiovascularly, the dog had intermittently weak femoral pulses, a sinus arrhythmia, poorly filled jugular veins and prolonged capillary refill time. The presence of mild prominent peripheral lymph nodes, serous ocular discharge and seromucoid nasal discharge, was also noticed. The abdominal palpation and rectal toucher were normal. The skin had an erythematous aspect and there was mild to severe alopecia on the neck, the ventral abdomen, the genital region and it was expanding distally and medially to the legs (Figures 1 and 2). On neurological examination, no abnormalities were present besides the abnormal gait and lethargy. There were no indications for neck, back, bone, muscle and/or joint pain. Considering the prolonged and multiple vague complaints and minimal abnormalities on neurological examination, metabolic problems needed to be considered first.

The last bloodwork dated from one month before admission, and therefore, a complete blood examination was repeated (Table 2). The CBC showed mild non-

Figure 1. The dog at the day of presentation. Dull and depressive expression. Alopecia in the neck region, in the inguinal region and at the hind legs. General poor skin- and coat quality.

regenerative microcytic anemia and mild thrombocytopenia. The serum biochemistry revealed mild azotemia and mildly increased creatine kinase. The serum electrolytes were within the reference interval. Because of the weak femoral pulses, the poorly filled jugular veins and prolonged capillary refill time, an echocardiography and electrocardiography (ECG) were performed. On echocardiography, there was a mild regurgitation at the level of the mitral- and tricuspid valve. The ECG performed during echocardiography showed a sinus arrhythmia with occasional supraventricular premature complexes and a heart frequency of 80 bpm. The fractional shortening was borderline low (27% (30-35%)). Thoracic radiographs showed a mild diffuse broncho-interstitial pattern, probably related with the slightly increased body condition of this dog. Because of the ongoing gastrointestinal complaints, the abdominal ultrasonography was repeated, revealing bilateral small adrenal glands but no further abnormalities. Special attention was paid to signs of vascular thrombosis, considering the weakness, the stiff gait and the weak femoral pulses, but these were not present. Urine was collected by ultrasound-guided cystocentesis and complete urinalysis only revealed urine specific gravity of 1.018.

Because of the subnormal fractional shortening, the bilateral alopecia and depressive behavior, HT was an important differential diagnosis but could not explain all clinical signs nor clinicopathological abnormalities. Secondly, based on the sustained vague and non-specific complaints, the bilateral small adrenal glands, the absence of a stress leukogram, the recurrent azotemia and non-regenerative microcytic anemia, HA was also considered to be an important possibility. Therefore, canine total thyroxine (TT4) and thyroid-stimulating hormone (TSH) concentrations were measured and an ACTH-stimulation test was performed (Table 2). Before executing the ACTH-stimulation test, all referring veterinarians were contacted to confirm that no corticosteroids had been given to the dog in the last two months since these could influence the results of the ACTH-stimulation test. The decreased TT4 and increased TSH concentrations were consistent with a diagnosis of primary HT. Additionally, the ACTH-stimulation test revealed HA. A diagnosis of atypical HA was made considering that the serum concentrations of sodium and potassium were within normal limits. To differentiate between atypical primary or secondary HA, serum aldosterone concentrations were measured before and after ACTH-stimulation. Both were very low, consistent with primary HA (Table 2).

Initial treatment consisted of prednisolone and levothyroxine. The prednisolone (Prednisolone, Kela Laboratories, Sint Niklaas, Belgium) was started at 0.14 mg/kg, twice a day (BID) and was gradually tapered based on the clinical improvement of the dog and the known side-effects of prednisolone (Table 3). The initial dose of levothyroxine (Forthyron, Eurovet Animal Health BV, Bladel, the Netherlands) was 0.002 mg/kg BID and this was increased every two weeks up to 0.008 mg/kg BID (Table 3). The levothyroxine supplementation was started ten days after the prednisolone supplementation and at a very low dose because otherwise an Addison crisis may be induced (Scott-Moncrieff, 2015b). Two weeks after diagnosis, the dog's clinical condition had improved markedly

Figure 2. The affected genital region at the day of presentation. General poor skin- and coat quality and severe alopecia in the genital region.

Table 2. Complete blood count, serum biochemistry profile, electrolyte concentrations, hormonology. Day 0 is the day of admission at the Faculty of Veterinary Medicine.

Variable		Units	Day 0	2 weeks	1 month	2 months	3 months	6 months	9 months	Reference interval
CBC										
	HCT	%	30.2	26.7	31 *	38.1	/	/	50.1	37.3-61.7
	Hemoglobine	g/dl	11.2	10.1	/	13.6	/	/	17.8	13.1-20.5
	MCV	fL	56.6	57.9	/	62.4	/	/	64.9	61.6-73.5
	MCH	pg	21	21.9	/	22.3	/	/	23.1	21.2-25.9
	Reticulocyte	K/µL	18.2	29.5	/	63.5	/	/	42.5	10-110
	WBC	109 g/L	9.3	11.6	/	10.28	/	/	8.09	5.05-16.76
	Neutrophils	109 g/L	3.6	7.66	/	7.51	/	/	6.35	2.95-11.64
	Lymphocytes	109 g/L	4.76	3.08	/	2.18	/	/	1.25	1.05-5.1
	Monocytes	109 g/L	0.32	0.65	/	0.42	/	/	0.35	0.16-1.12
	Basophils	109 g/L	0	0	/	0.01	/	/	0.01	0-0.1
	Eosinophils	109 g/L	0.62	0.07	/	0.16	/	/	0.13	0.06-1.23
	TBC	K/µL	135	372	/	329	/	/	212	148-484
Biochemistry										
	Urea	mmol/L	13.7	/	/	/	/	/	7.1	2.5-9.6
	Creatinine	µmol/L	227	/	/	/	/	/	152	44-159
	Total protein	g/L	64	/	/	/	/	/	67	52-82
	Albumin	g/L	26	/	/	/	/	/	37	23-40
	Globulines	g/L	38	/	/	/	/	/	30	25-45
	Glucose	mmol/L	3.89	/	/	/	/	/	6.00	3.05-4.99
	ALT	U/L	61	/	/	/	/	/	93	10-100
	ALP	U/L	19	/	/	/	/	/	78	23-212
	CK	U/L	454	/	/	/	/	/	/	10-200
Electrolytes										
	Sodium	mmol/L	152	156	157	155	158	158	158	144-160
	Potassium	mmol/L	5.4	5.5	4.6	4.8	4.2	4.2	4,1	3.5-5.8
	Chloride	mmol/L	120	116	117	118	119	120	118	109-122
	Calcium	mmol/L		2.51	/	/	/	/	2.50	1.98-3
	Phosphorus	mmol/L		1.61	/	/	/	/	1.03	0.81-2.2
Hormonology										
	TT4	nmol/L	<6.45	16.77	/	52.89	/	/	43.86	6.45-43.86
	TSH	ng/ml	6.43	/	/	/	/	/	/	
	ACTH stim. Test	nmol/L								
	Cortisol pre		< 3	/	/	/	/	/	/	
	Cortisol post		< 3	/	/	/	/	/	/	
	Aldosterone	pmol/L	<27.74							

HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, WBC: white blood cells, TBC: thrombocythes ALT: alanine transferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, CK: creatinine kinase, TT4: total thyroxine, TSH: thyroid stimulating hormone, ACTH: adrenocorticotropic hormone. * means: microhematocrit or packed cell volume. Increased values are colored in red, decreased values are colored in green.

(e.g. less depressed, able to make long walks, good appetite, no more nausea, etc.). Nevertheless, there were new complaints of polyuria and polydipsia (pu/pd). The coat and skin quality was still poor and even worsened at the beginning of therapy. There were complaints of excessive hair loss and cold intolerance (shivering) (Figure 3). Laboratory tests showed a normalized TT4 value and stable electrolyte values, but a moderate microcytic anemia persisted (Table 2). Because of pu/pd, the prednisolone dose was tapered to 0.1 mg/kg BID and two weeks later to once a day (SID) (Table 3). Follow-up appointments were scheduled after one, two, three, six and nine months. The general condition of the dog kept improving and was considered normal after two months. During follow-up, no new abnormalities were noticed on physical examination. Three weeks after diagnosis, all cardiovascular parameters (heart rate, capillary refill time, pulse quality) normalized. However, on follow-up, ECG a lone atrial fibrillation (LAF) with a ventricular response rate of 104 beats per minute was noticed. No underlying structural cardiac disease could be identified. The first improvement of coat quality and quantity was seen after two months. After nine months, the haircoat completely recovered (Figure 4). Blood examinations were frequently repeated during a

Figure 3. The dog one and a half month after initiation of therapy. Worsening of skin- and coat quality and the alopecia is more severe and more generalized. At that time, the dog suffered from excessive hair loss and cold intolerance.

Figure 4. The dog nine months after initiation of therapy. The skin- and coat quality is completely normalized.

nine-month follow-up period showing normalization of all initial abnormalities. Serum electrolytes always remained within normal limits (Table 2). After nine months, a complete blood work was repeated, which revealed no abnormalities (Table 2). After one year, a control ECG did not show any more abnormalities either.

DISCUSSION

HA is a well-described endocrinopathy. Nevertheless, the atypical primary form is not that well known by practicing veterinarians and is more challenging to diagnose. In cases with a chronic history of waxing and waning complaints, without major abnormalities on blood examination, especially with an absent stress leukogram, which do not improve permanently on symptomatic treatment, the suspicion of HA should arise (Scott-Moncrieff, 2015a). In the present case, the dog had already been suffering from these vague complaints for more than a year. Several symptomatic treatments were given but gave only mild, temporary improvement. HA is mostly seen in young to middle-aged, female dogs; so the dog in this case fits with the typical signalment. Moreover, many of the abnormalities, which were seen on physical and blood examinations and on medical imaging (e.g. dull and depressive expression, weakness, intermittently weak femoral pulses, mild generalized lymphadenopathy, mild microcytic non-regenerative anemia, mild azotemia, bilateral small adrenal glands) were all findings that may be indicative for HA. An ACTH-stimulation test was performed after contacting all referring veterinarians to ensure no corticosteroids were given two months prior to admission. As this was not the case, the test was reliable and confirmative for HA. A less expensive test to rule out HA is the basal cortisol concentration (bcc) (Lennon et al., 2007). This test has a high negative predictive value (100%) and so, dogs with bcc's higher than 2µg/dL are highly unlikely to have HA (Lennon et al., 2007). It is important to realize that values below 2µg/dL are not sufficient to diagnose HA, and an ACTH-stimulation test is always required to confirm the diagnosis (Lennon et al., 2007). In the dog described, the bcc was not measured but an ACTH-stimulation test was immediately performed. After confirmation of HA, the next step was to differentiate between primary and secondary HA by evaluating endogenous ACTH or ACTH-stimulated aldosterone concentrations. In theory, the measurement of basal ACTH concentration is more accurate than measuring aldosterone concentrations to differentiate between primary and secondary HA (Scott-Moncrieff, 2015a). Unfortunately, plasma ACTH measurement is not practical, because specific and strict guidelines must be followed for sample collection and handling. Endogenous ACTH is very labile, it has a short half-life, is not thermostable and should not come into contact with glass (Behrend et al., 2013). In contrast, aldosterone is more stable, specific handling precautions are not needed and assays are more widely available (Sieber-Ruckstuhl, 2006). Therefore, it was decided to measure ACTH-stimulated aldosterone concentrations in the present case. Aldosterone secretion should be impaired in case of primary HA and should be normal with secondary HA. Dogs with atypical primary HA are suspected of having normal aldosterone concentrations due to the sparing of the zona glomerulosa (Baumstark et al., 2014). In this case, the decreased aldosterone concentrations before and after ACTH administration led to the diagnosis of atypical primary HA. These low aldosterone concentrations are contradictory to the normal sodium and potassium concentrations measured in this dog (Table 2). It has been assumed that normal aldosterone concentrations are mandatory to maintain normal electrolyte values as seen with primary atypical HA (Baumstark et al., 2014; Scott-Moncrieff, 2015a).

Table 3. Schematic representation of the posology of the initiated treatment after diagnosis.

Time	Drug	Time	Drug
	Prednisolone (mg/kg)		Levothyroxine(mg/kg)
D1	0.14 BID	D1	0.002 BID
D14	0.1 BID	D14	0.004 BID
D30	0.1 SID	D30	0.006 BID
		D 44	0.008 BID
D 60	0.1 SID	D 60	0.008 BID

D1: first day of treatment; SID: once daily; BID: twice daily

It is difficult to underpin this assumption because in most reported cases, aldosterone concentrations were not evaluated (Scott-Moncrieff, 2015a). However, in a recent study describing aldosterone concentrations in dogs with HA, four dogs with atypical primary HA had low baseline- and ACTH- stimulated aldosterone concentrations (Baumstark et al., 2007). These findings and the laboratory results of the present case, imply that there have to be other mechanisms which may explain the maintenance of normal sodium and potassium concentrations. Some of the possible hypotheses include increased dietary sodium intake, increased renin concentrations (compensated failure of the zona glomerulosa), partial sparing of the zona glomerulosa, mutation of the ACTH receptor gene, disparity in the rate of destruction of glucocorticoid and mineralocorticoid secreting cells and influence of concurrent disease such as HT (Oelkers, 1992; Frank et al., 2013). In this case, the owner was not aware of a higher sodium intake and the dog had been partially anorectic for a long time. Other hypotheses, and particularly the influence of concurrent HT, could not be excluded. Nonetheless, it can be concluded that the appearance of normal sodium and potassium values does not always reflect a normal function of the zona glomerulosa and that further research on large scale is required.

In this case, a concurrent diagnosis of primary hypothyroidism (HT) was made, based on low TT4 and high TSH concentrations. When the dog became ill, that was forty-five weeks before admission, with complaints of partial anorexia, vomiting, diarrhea and weakness, blood examinations were performed and showed increased canine TT4 value (Table 1). This increased TT4 value may have been caused by anti-thyroid hormone antibodies (Thacker et al., 1992). These antibodies may occur in 2% of dogs with clinical signs of lymphocytic thyroiditis and in 15% of dogs with HT (Nachreiner et al., 2002). Anti-thyroid hormone antibodies and TSH were not measured in this dog. Therefore, it is uncertain if the dog had already been suffering from clinical HT at that time point. Nevertheless, it was certainly suffering from a polyendocrine disorder at the day of presentation. The developing time between the first and the second endocrinopathies may vary with an average of four months (Blois, 2011). In this dog, both diseases were diagnosed simultaneously, so it was impossible to determine which disease had developed first. The appearance of multi-endocrinopathy is rare in the dog, with a prevalence of only -5% of the dogs with HA (Peterson 1996; Scott-Moncrieff, 2015a). An immune-mediated etiology is suspected in these polyglandular syndromes (Scott-Moncrieff, 2010; Blois, 2011; Scott-Moncrieff, 2015a; Scot-Moncrieff, 2015b). The most common combinations of immune-mediated endocrine disorders in dogs with multiple endocrinopathies have been reported to be HT and diabetes mellitus (28%) and HT and HA (23%) (Lathan and Tyley, 2005; Blois, 2011). The presence of multiple endocrine disorders in dogs resembles polyglandular deficiency syndrome in humans, which is further divided in three types (Neufeld et al., 1980). The combination of primary HA, primary HT and/or insulin-dependent, diabetes mellitus is known as type II polyglandular autoimmunity or Schmidt's syndrome. This syndrome has already been described (18 cases) in small animal veterinary medicine (Bowen et al., 1986; Bartges and Nielson, 1992; Kooistra et al., 1995, Lifton et al., 1996; Melendez et al., 1996, Smallwood and Barsanti, 1995; Adissu and foster, 2015). In ten of the eighteen reported cases, an atypical HA was described in combination with HT. The diagnosis of a polyglandular syndrome may be assumed by demonstrating circulating antiadrenal and anti-thyroid antibodies or by histology of glandular biopsies (Blois et al., 2011). One canine case with both anti-adrenal and anti-thyroid antibodies has been reported (Bowen et al., 1986). In two cases, autopsy and histology revealed an immune-mediated glandular destruction (Kooistra et al., 1995; Adissu and Foster, 2010). In the present case, antibodies were not measured. Since the dog was doing perfectly well, biopsies for histology were not advised. The signalment, clinical history, the abnormalities found on physical and blood examinations (typical of primary HA and primary HT) and on medical imaging in combination with the immediately positive response to treatment all indicated polyendocrinopathy, more specifically the Schmidt's syndrome.

The treatment of atypical HA, without electrolyte abnormalities, initially consists of the supplementation of glucocorticoids alone (0.1 to 0.22 mg/kg/day prednisolone) (Scott-Moncrieff, 2015a). The therapy should lead to rapid, total, clinical improvement, and the dose should be gradually tapered to maintenance rate (Scott-Moncrieff, 2015a). Previously, it had been assumed that the supplementation of mineralocorticoids was unnecessary because the function of the zona glomerulosa, which produces mineralocorticoids, was thought not to be affected (Kintzer and Peterson, 1997; Scott-Moncrieff, 2015a). However, knowing that serum aldosterone concentrations do not always remain within normal limits, this theory is questioned (Baumstark et al., 2014). In human medicine, the decision to supplement mineralocorticoids or not is based on the measurement of renin concentrations (Stewart and Krone, 2011). In veterinary medicine however, the measurement of renin concentration is not widely available. Therefore, it is often advised to only supplement glucocorticoids. Moreover, rapid and complete clinical improvement is seen in dogs treated with only glucocorticoid supplementation. In addition, drugs for mineralocorticoid supplementation might be costly and not always easily available (Baumstark et al., 2014; Scott-Moncrieff, 2015a). In the present case, prednisolone therapy was started at 0.14 mg/kg BID and gradually tapered, because of pu/pd, until 0.1 mg/kg SID (Table 3). After two weeks of therapy, the dog was already doing much better and after eight weeks, the general condition of the dog was considered normal. Nonetheless, at the first recheck appointment, a lone atrial fibrillation (LAF) was noticed on the control ECG. This LAF was not present on the day of admission. LAF may be associated with endocrinopathies, such as (atypical) HA and HT, although a cause-and-effect relationship has not been clearly established yet (Gerritsen et al., 1996; Takemura et al., 2002; Riesen and Lombard., 2006). In this case, it was difficult to determine which one of the two endocrinopathies had been the possible cause of the LAF. However, HT seems to be more likely because the treatment for HT had only been started since one week, and the HT had not yet been controlled at the time of LAF diagnosis. One year later the clinical and laboratory findings were completely normalized and LAF was not present anymore.

A good follow-up of dogs with atypical primary HA is mandatory. Blood examinations have to be re-evaluated regularly because of the risk of progression to complete adrenal failure (Scott-Moncrieff, 2015a). The destruction of the adrenal cortex is thought to be caused by an immune-mediated adrenalitis, which may progress to the zona glomerulosa of the adrenal cortex (Schaer et al., 1986; Lifton et al., 1996; Lathan and Tyley, 2005). This progression leads to complementary mineralocorticoid deficiency, and additional supplementations with mineralocorticoids to the prednisolone supplementation will be required (Scott-Moncrieff, 2010). However, this progression is difficult to predict in the individual patient. Some dogs do not progress during years of monitoring and others suddenly do (Lifton et al., 1996; Thomson et al., 2007; Scott-Moncrieff, 2010; Baumstark et al., 2014). A retrospective case study showed 12.5% chance of progression (lifton et al., 1996). Moreover, most of the dogs that progress, do so within the first year after diagnosis (Rogers et al., 1981; Lifton et al., 1996a normal Na:K ratio (> 27; Thompson et al., 2007). Therefore, electrolyte concentrations should be monitored every one to three months for at least the first year after diagnosis (Kintzer and Peterson, 1997; Scott-Moncrieff, 2010; Scott-Moncrieff, 2015a). Owners of these dogs should adequately be informed, so they can recognize the first clinical signs of mineralocorticoid deficiency and contact the veterinarian (Baumstark et al., 2014), because rapid supplementation of mineralocorticoids may be of vital importance (Scott-Moncrieff, 2015a). The dog in this case was regularly reevaluated for more than a year but the electrolytes always stayed within normal limits.

In addition to the HA, this dog was also diagnosed with HT and thus with a polyglandular syndrome. In these cases, it is important to treat every glandular deficiency separately (Baumstark et al., 2014). Although an immune-mediated etiology is suspected, it is not advised to start immunosuppressive drug therapy in these syndromes (Scott-Moncrieff, 2015a). Furthermore, it should be borne in mind that the treatment of one glandular deficiency might influence the other. For example, supplementation with thyroid hormones in untreated Addison patients, may precipitate an Addison crisis because the normalization of the slower metabolism associated with HT leads to increased corticosteroid demands (Bowen et al., 1986; Scott-Moncrieff, 2015a). In the present case, a very low dose of levothyroxine was therefore started only ten days after the prednisolone supplementation was initiated. The levothyroxine dose was gradually increased to avoid worsening of the cardiac problems and HA (Table 3). Although the dog was doing clinically better within short term, initially, the skin and coat problems worsened and it took nine months before skin and coat could be considered normal again. Nonetheless, this response and reaction to levothyroxine therapy can be considered normal for a hypothyroid dog. It has been documented that initially, the hair coat may appear to worsen (Credille et al., 2001), and that it may take several months of therapy for complete regrowth and progression of skin quality (Scott-Moncrieff, 2015b).

CONCLUSION

The dog of the present case was suffering from waxing and waning, vague complaints for more than a year before it was diagnosed with atypical primary HA and primary HT. Atypical HA is more challeng-

ing to diagnose than typical HA, since the typical abnormal sodium-to-potassium concentration ratio is absent. This emphasizes the fact that normal serum electrolytes are not a reliable parameter to exclude HA. Moreover, in case of a dog with a chronic history of vague complaints, with an absent stress leukogram and which does not react properly on symptomatic treatment, high suspicion should arise for (atypical) HA. An unexpected finding in this case was the decreased aldosterone concentrations despite the normal electrolytes, as described in other cases. Further research in atypical HA patients is required.

In addition, the dog was also diagnosed with primary HT. The polyendocrinopathy, which was seen in this dog has seldom been described in veterinary medicine and is similar to a polyglandular syndrome type II (Schmidt's syndrome) described in humans.

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Solid feed provision reduces fecal clostridial excretion in veal calves

Het geven van vaste voeding aan witvleeskalveren vermindert de uitscheiding van clostridia in de mest

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ABSTRACT

Enterotoxemia is characterized by a highly fatal hemorrhagic enteritis in cattle, caused by *Clostridium perfringens*. Production systems with intensive feeding, such as the veal industry, are predisposed. The primary objective of this study was to determine the effect of solid feed provision on fecal *C. perfringens* excretion in veal calves. Ten Holstein Friesian bull veal calves were randomly assigned to one of two test diets. Group 1 received solemnly milk replacer twice daily, while group 2 received milk replacer and a maximum of 300g solid feed/day, consisting of a mixture of 30% barley, 30% corn, 30% hulled wheat and 10% chopped straw. The number of *C. perfringens* per g feces or fecal clostridial counts (FCC) were determined for all calves. Mean FCC were significantly lower in the calves fed milk replacer and solid feed, than in the calves fed solemnly milk replacer. Although the correlation between FCC and enterotoxemia risk remains to be determined, the provision of solid feed to veal calves reduced clostridial excretion, which might contribute to the prevention of this disease.

SAMENVATTING

Clostridium perfringens geassocieerde enterotoxemie is een belangrijke oorzaak van sterfte bij kalveren in intensieve opfoksystemen. Het doel van deze studie was om het effect van vaste voeding te beoordelen op de uitscheiding van *C. perfringens* in de mest bij witvleeskalveren. Twee verschillende testdiëten werden at random toegewezen aan tien holsteinfriesian-witvleeskalveren. De kalveren in groep 1 werden uitsluitend gevoed met melkpoeder, terwijl kalveren in groep 2 zowel melkpoeder als een maximum van 300g vaste voeding per voederbeurt kregen. De vaste voeding bestond uit 30% gerst, 30% mais, 30% tarwe en 10% gehakseld stro. Vervolgens werd het aantal clostridia in de mest bepaald. Kalveren die zowel melkpoeder als vaste voeding kregen, scheidden significant minder clostridia uit in de mest dan kalveren die uitsluitend met melkpoeder werden gevoed. Er moet nog verder onderzocht worden of het verschaffen van vaste voeding aan witvleeskalveren een preventief effect heeft op enterotoxemie.

SHORT COMMUNICATION

Enterotoxemia (hemorrhagic enteritis) is a highly fatal disease in cattle, caused by *Clostridium perfringens*. The disease predominantly affects calves in intensive production systems, such as the veal industry or feedlots (Griner and Bracken, 1953; Niilo et al., 1974, Glock and Degroot, 1998; Muylaert et al., 2010). In Belgian blue veal calves, enterotoxemia accounts for 20% of the total mortality rate (5-7%) and occurs predominantly at the end of the production cycle when animals have a high economic value (Pardon et al., 2012). Given the high fatality rate and rapid course of the disease, control is mainly focused

on prevention. It is generally accepted that especially dietary factors, such as high protein diets are associated with the enterotoxemia risk (Lebrun et al., 2010). Therefore, dietary adaptations might help in the prevention of the disease. Traditionally, white veal calves are not fed any solid feed in order to maintain the desired pale meat color. In recent years, several studies have demonstrated beneficial effects of the provision of solid feed on ruminal development, daily growth and animal welfare, without negative effects on meat color (Suarez et al., 2007; Webb et al., 2013). Moreover, for welfare reasons, European legislation obliges a minimum provision of solid feed (European Council Directive 2008/119/EC). It is hitherto unclear what the consequences of solid feed provision are with respect to the risk of developing enterotoxemia. It is believed that fiber-rich feeds inhibit clostridial overgrowth, by altering the intestinal microbiota (Zhang et al., 2013). Thus, the hypothesis of the present study was that solid feed provision would reduce the fecal excretion of *C. perfringens* in veal calves.

To determine the effect of solid feed provision on fecal *C. perfringens* excretion a cross-sectional study was conducted on a commercial white veal farm (5000 animals present). To determine a one log difference between test groups, with a standard deviation of 40 CFU (colony forming units)/g feces, 95% confidence and 80% power, a sample size of 5 animals per test group was required. All procedures were approved by the local ethical committee (EC2014/016). Ten male Holstein Friesian calves (mean body weight= 52kg; mean age=14 days) were randomly assigned to one of two test groups (group 1 and group 2) on the day of arrival at the fattening unit. All calves were kept on a slatted floor without bedding. Calves were not vaccinated against Clostridia. However, both groups received amoxycilline in the first ten days after arrival at the fattening unit as a preventive start-up treatment. Both groups were fed the same amount and concentration of a commercial milk replacer (Nil product, Vilatca nv, Geel, Belgium), twice daily. The milk replacer provision increased from 220g DM (dry matter)/day on arrival to 750g DM/day at the age of three months. The milk replacer contained 8% ash, 17.7% crude fat and 18% crude protein (CP) (whey, vegetal protein, no casein) per feeding. Group 2 received solid feed once daily in addition to the milk replacer, starting with 32g FP (fresh product)/feeding gradually increasing to 300g FP/feeding at the age of three months. The solid feed consisted of a mixture of whole grains (barley (30%), corn (30%), hulled wheat (30%)) and 10% chopped straw (ash 2.4%, CP 9.6%).

Calves were sampled at the age of three months. Fecal samples were taken from each calf three times per day (in the morning shortly after feeding around 8.00h, at midday halfway between feedings around 13.00h and in the evening shortly before feeding around 18.00h). All fecal samples were taken with rectal gloves directly from the rectum, and cooled to 4°C until processing within 24 hours. Each sample was plated nine times (technical repeats). Quantification of *C. perfringens* was performed as follows. One gram of the fecal samples was suspended in 9 mL of PBS (phosphate buffered saline). Ten-fold dilutions were made in sterile PBS. Subsequently, for each dilution, six droplets of 20 µL were plated on Columbia agar (Oxoid, Basingstoke, UK) with 5% defibrinated sheep blood, containing 12 mg kanamycin sulphate and 30,000 U/l polymyxin B sulphate. Bacteria were allowed to grow overnight at 37°C in an anaerobic cabinet (Ruskinn Technology, Bridgend, UK) with 84% N_2, 8%CO_2 and 8% H_2. The identification of *C. perfringens* colonies was made on the basis of a dual hemolysis zone. The number of CFU of *C. perfringens* per g feces (= fecal clostridial count (FCC)) was calculated based on the recovered colonies on the plates. To determine the effect of the diet on the FCC, a linear mixed model with repeated measurements was built with clostridial count (in CFU/g feces) as outcome variable. For each calf, three observations were added, one at each time point (morning, midday and evening). Each observation consisted of the mean of nine FCC performed on each sample. The outcome variable was tested for normal distribution. Time (morning-midday or evening) was added as a repeated measurement and a variance components covariance structure was used. Calf was added as a random effect to account for clustering of samples within a calf. Feeding regime (Group 1 or 2) was added as a fixed factor. Significance was set at $P<0.05$. All analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC), using the PROC MIXED procedure.

Mean±SD FCC was 505±1059 CFU/g feces and 56±119 CFU/g feces in groups 1 and 2, respectively. The effect of the test group was significant at every time point ($P<0.001$). The time effect (8.00h, 13.00h, 20.00h) was not significant (Figure 1).

The study of risk or protective factors for enterotoxemia is seriously hampered by the fact that this is a rare event disease (Lebrun et al., 2010). Unrealistically large sample sizes need to be taken into account

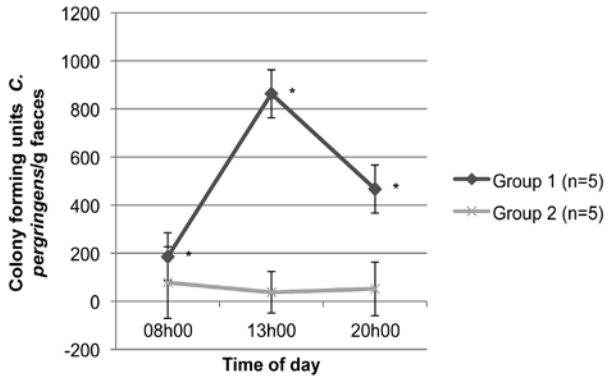

Figure 1. Evolution of the mean fecal clostridial counts (CFU/g feces) in veal calves receiving only milk replacer (group 1) or milk replacer and solid feed (group 2). Error bars represent the standard deviation; * indicates a significant difference (P<0.001) between the two groups at that time.

when looking at mortality due to enterotoxemia as the outcome variable. Therefore, alternatively, the effect of solid feed provision on fecal *C. perfringens* excretion was determined. The authors want to stipulate that the observations made in this study refer to FCC. Evidencing a direct link with enterotoxemia was not the objective. Higher FCC suggests an increased clostridial load in the intestinal microbiota, which may contribute to a higher risk of *C. perfringens* induced enterotoxemia. However, there is no known FCC threshold that would result in enterotoxemia.

The present study showed that supplying solid feed decreases fecal clostridial excretion in veal calves. The mechanism behind this effect is not completely understood. Veal calves fed exclusively milk replacer only develop a rudimental rumen (Suarez et al., 2007). In the veal calves offered solid feed, the limited amount of 300g per feeding even induced relevant ruminal development and an active ruminal function (Webb et al., 2013). This might lead to an intestinal environment less favorable for Clostridia, possibly favoring bacteria with a direct *C. perfringens* growth-inhibiting effect, such as for example *Bacillus subtilis* (Jeong and Kim, 2014). Fiber-rich feeds indeed do increase the proportion of *Bacillus spp.* in the ruminal microbiota, potentially inhibiting clostridial overgrowth (Zhang et al., 2013). Moreover, veal calves fed milk replacer and solid feed have an improved ruminal digestibility (Labussiere et al., 2009), potentially leading to a decreased availability of nutrients in the lower intestine, and thus leading to a lower FCC. Although the association between FCC and enterotoxemia risk remains to be determined in calves, the provision of solid feed to veal calves might be a useful preventive tool against enterotoxemia. This opens perspectives for further dietary management of the disease. It remains to be determined whether the composition of the solid feed can be optimized in order to reduce FCC even further.

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Al of niet bedwelmd slachten en offeren: wat er achter de woorden schuilt

To slaughter, to sacrifice: the historic background of killing animals for food

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SAMENVATTING

De term 'slachten' is afgeleid van slaan, knock-out slaan. Bewusteloos slaan was een primitieve, nogal drastische vorm van bedwelming voor het slachten van vee. Dat werd steevast gevolgd door het eigenlijke doden met verbloeden door halssnede of doorsnijden van de grote bloedvaten bij het hart. De moderne varianten met schiettoestellen en metalen pennen of met elektrische bedwelming verschillen niet wezenlijk van het 'slaan' met de hamer. Gewezen wordt op het belang van deze methode als basis van het onderscheid met ritueel slachten in de joodse traditie en ook met het 'nekken' (doorsnijden van het ruggenmerg in de nek) zoals in grote delen van de wereld gebruikelijk bij rundvee, bij ons enkel bij kleinvee (kippen, konijnen, duiven). Bij een tweede belangrijke vorm van ritueel slachten, voor de productie van vlees toegelaten (halal) voor moslims, is niet de manier van slachten essentieel, wel een minstens rudimentair ritueel van offeren aan Allah. Bedwelmen kan ook voor halalvlees, mits het dier niet doodgeslagen wordt en goed uitbloedt. Dit wordt tot op heden echter slechts door een minderheid van islamgeleerden aanvaard.

Een korte beschrijving wordt gegeven van de achtergrond van de voorschriften, die vermoedelijk te zoeken zijn in de oertijden van de veehouderij, toen het slachten van een dier bij herdersvolkeren nog iets zeer uitzonderlijk was, iets wat zelfs de plaats kon innemen van het mensenoffer aan God, zoals het bijbelverhaal van Abraham illustreert. In de christelijke traditie gebeurde dat door de offerdood van Jezus, zoon van God, het Lam Gods, dagelijks herhaald in het misoffer. Dat offer betekende een radicale breuk met de joodse voedselvoorschriften. Het verklaart niet enkel de afwezigheid van rituelen bij het slachten zoals bij ons gebruikelijk, maar ook het stilaan verdwijnen van op religie gebaseerde voedseltaboes na de eerste eeuwen van het christendom.

ABSTRACT

'Slaughter' and 'slay', words of Germanic origin, and 'beat' and 'abattoir' of Latin descent, all refer to a primitive way of rendering animals unconscious, of 'knocking them out', before actually killing them with a knife by cutting the throat or the main blood vessels in the heart region. The situation is complicated by religious traditions. Ritual slaughter in the Islamic and Judaic traditions dates back to Biblical times, when Abraham (Ibrahim) was prevented by God (Jahweh, Allah) from offering (sacrificing) his only son, who was replaced on the altar by a ram. In the orthodox Jewish tradition, the killing of animals for food is complicated by a strong taboo against blood. This came to expression in the strict rules for killing the conscious animal with a sharp knife and for avoiding contact with the animal's blood. In the Christian tradition, the taboos disappeared after the early period because it was realized that Jesus, as the Lamb of God, has sacrificed himself in order to save and redeem mankind. The notion of sacrifice is still associated with killing animals for food or other human use. In the biomedical literature, the term 'sacrifice', originally meaning 'offer', is frequently used to designate the killing of experimental animals. In four surahs in the Koran, the importance is stressed of offering all animals being put to death for food to the One and Only Allah. The slaughtering technique is not stipulated in any further detail, except for the rule that the animals should not be beaten to death and that the blood evacuated should not be consumed.

INLEIDING

Actueel is er heel wat te doen over het al dan niet bedwelmen van slachtdieren. De actuele discussie gaat daarbij nog over de tijd dat het dier nog pijnbeleving kan hebben bij het bedwelmen. Hoe zit dat bij vroegere en huidige methoden? Om die discussie over het al of niet bedwelmen enigszins te begrijpen moeten we ver terug in de tijd naar de vroege veehouderij, toen het slachten van een dier een zeldzaam en plechtig moment was. Enkele Hebreeuwse bijbelepisoden moeten in die tijden ontstaan zijn. Denk aan het verhaal van de 'verloren zoon'. Bij diens onverhoopte terugkeer laat de dolgelukkige vader 'het beste kalf slachten'. De lange tijd nomadische Hebreeuwse herdersstammen achtten de veeteelt hoog. Daarvan getuigt het verhaal van Kaïn en Abel, waarin de 'zachtzinnige' herder Abel gedood wordt door zijn landbouwende broer Kaïn omdat God, Jahweh, de voorkeur gaf aan een lam, geschenk van Abel.

Schaap en rund, de in de context van dit artikel belangrijkste huisdieren, werden in de beginfase van hun domesticatie gehouden omwille van het vlees (1). Het was een aanvulling en een surrogaat voor het wildvlees. De dieren trokken mee met de nomadische jagers als levende vleesvoorraad. Pas later veranderde dat en werden meeste huisdieren verder gedomesticeerd, vooral omwille van de opbrengst aan melk, en niet minder belangrijk, om ploegen en karren te trekken en om lasten te dragen (trek- en lastdieren). Wol was een nevenproduct, net als leder, hoorn en andere niet-eetbare lichaamsonderdelen.

In tegenstelling tot wat bij jagersstammen het geval is, was het doden van een dier bij de verder ontwikkelde herdersvolkeren, zoals ook de joodse stammen in de beginperiode van hun in de Thora opgetekende geschiedenis, eerder een noodzakelijk kwaad, waartegen zelfs een zekere weerstand bestond (2). Wel moesten de meeste bokjes en rammen, ongeveer even talrijk geboren als vrouwelijke dieren, geslacht worden. Die geven immers geen melk. Castreren gebeurt vermoedelijk al heel lang, maar dat leverde een economisch minder waardevol alternatief op. Het was eerder een noodgreep, een middel om de al te heftige, mannelijke dieren te laten bedaren, zodat ze konden 'gevet mest' worden en als 'leverancier' van vlees toch nog opbrengen voor hun eigenaars.

Het is in deze context dat we wat hier volgt moeten bekijken (3). Eerst wordt een kort overzicht gegeven van de oeroude slachttechnieken, waarvan sommigen een primitieve manier van bedwelmen inhouden. Vervolgens proberen we de eveneens duizenden jaren oude sfeer van offeren te begrijpen, waarin het slachten plaats vond. Aan het einde van deze bijdrage zien we waarom in de christelijke traditie, die we in Europa en de door Europeanen gekoloniseerde werelddelen kennen, de sfeer van offeren verdween, samen met de vele voedseltaboes.

SLAAN

We staan er niet bij stil, terwijl het toch evident is, het woord slachten is afgeleid van slaan. Bewusteloos slaan is een primitieve vorm van bedwelming (verdoving). Dit brengt ons naar de verschillende, van ouds gebruikte vormen van slachten in diverse werelddelen, al dan niet religieus ingebed. De moslimpraktijk die een rudimentair offerritueel inhoudt, voert ons terug naar de begintijd van de veehouderij, toen een dier slachten nog een plechtig en zeldzaam gebeuren was.

Eeuwenlang ving het slachten van runderen in onze streken aan met een hamerslag op het voorhoofd, zoals Figuur 1 te zien geeft. De kop en/of de ledematen van het dier werden geïmmobiliseerd, de man met de hamer, letterlijk de 'slager', gaf één slag en het dier zeeg groggy neer. Op dat letterlijk 'neerslaan' van het dier volgde de eigenlijke 'doodsteek' (geen gratuite term) in de aorta. Die manier van 'de kop inslaan' was regel bij zware varkens en runderen, behalve bij de gemakkelijker te overmeesteren en dus minder gevaarlijke kalveren waarvan de kop verkocht werd als delicatesse.

Zo ging het er aan toe op de vele primitieve slachtvloeren van de slagers, op achterkoertjes en dergelijke, her en der in stad en op het platteland. Zo gebeurde het ook nog lang nadat de overheden vanaf ongeveer halverwege de jaren 1800 om hygiënische redenen slagers verplichtten hun activiteiten te verleggen naar gemeentelijke slachthuizen. Zo ook in privéslachthuizen zoals in de reusachtige vleesbedrijven in Chicago, waar er op onvergelijkelijk veel grotere schaal geslacht en verwerkt werd. Upton Sinclair hing daar in 1906 een hallucinant beeld van op in zijn destijds ophefmakende boek 'The Jungle' (4). Ook daar werd er in essentie op dezelfde manier te werk gegaan. De 'meppers' waren gewapend met hamers. Ze 'sloegen' in snel tempo de runderen die elk apart gevat stonden in hekkens op een verhoogde vloer. De knock-out geslagen dieren gleden vervolgens nog stampend naar de 'stekers' toe. Die dienden de eigenlijke 'doodsteek' toe, een forse messteek vlak bij het hart. Alle werklui hadden een beperkte taak, een voorafspiegeling van het lopende bandsysteem in de autoconstructieateliers van Henry Ford en anderen in Detroit, niet zo ver daar vandaan.

Schedelletsels als gevolg van 'slaan' zijn in onze streken archeologisch gedocumenteerd vanaf de Romeinse tijd (5). Het onmiddellijk gevolg van dat slaan is niet de dood, maar bewusteloosheid te vergelijken met de knock-out bij het boksen. Het centrale zenuwstelsel ondergaat een plotse heftige drukverandering. Het dier valt ogenblikkelijk bewusteloos neer. Het hart pompt nog, ook als de ademhaling ophoudt. De poten trekken heen en weer. Met elektrocutie of CO_2-toediening kan een gelijkaardig bewustzijnsverlies bereikt worden.

Vanuit een eeuwenoude gewoonte en vermoe-

Figuur 1. Prent van Moreau le jeune (1765, detail).

Figuur 2. Illustratie uit Rühl, J. (1914). Notice sur l'abattoir de Bruxelles. Exposé sur quelques réformes en matière d'abattage. In: *Nos Meilleurs Amis*, jg. 22 nr. 6, p. 63. Dit was het tijdschrift van de Brusselse vereniging voor dierenbescherming die deze manier van 'slaan' propageerde.

Figuur 3. Slagtoestel voor pen ingeslagen met hamer: zie Figuur 2 (Museumcollectie Diergeneeskundig Verleden Merelbeke, UGent).

Figuur 4. 'Schermer' schiettoestel met patronen (Museumcollectie Diergeneeskundig Verleden Merelbeke, UGent).

delijk omwille van de praktische voordelen die het werken met een bewusteloos dier biedt, 'sloeg' men voorafgaand aan het eigenlijke doden. 'Slaan' was essentieel, althans bij ons en in de ons omringende West-Europese landen. In alle Germaanse talen vinden we dat woord terug in de bij het doden van dieren voor consumptie gebruikelijke terminologie: slachten, slachthuis, enz., afgeleid van 'slaan'. Denk ook aan het Franse abattre en abattoir, afgeleid van bâton (knots, stok). In Engeland bleef de lokale, grotendeels Germaans sprekende, Angelsaksische boerenbevolking varkens, runderen en schapen slachten ('slay, slaughter'), maar ze waren in 1066 verslagen ('they were beaten') door de geromaniseerde Normandiërs, die wisten wat 'battre' was en die hun nieuwe onderdanen ook wel eens durfden trakteren op stokslagen (beats). Vanzelfsprekend werd ook het militaire jargon van deze heren aan het Frans ontleend: 'battle' van 'bataille', 'battle field', enz., allemaal termen die teruggaan op de primitieve knots.

Over angst en pijn bij het slachten, twee heel verschillende psychische en fysische fenomenen, en over de manieren om die tegen te gaan, is al heel wat bekend, maar toch nog onvoldoende (6). Bij het verlies van bewustzijn, onafhankelijk van de manier waarop dit gebeurt, verdwijnen zowel angst als pijngevoel. In Nederland en in Belgische wetteksten wordt de term 'bedwelmen' gebruikt. 'Dwelm' is een Middelnederlands synoniem van 'zwijm'. In het Vlaamse taalgebruik overheerst 'verdoven'. De onderzoekers De Tavernier en Aerts, gespecialiseerd in dit onderwerp, menen dat 'bedwelmen' een correctere term is, want het is de bedoeling de dieren buiten bewustzijn te brengen en niet enkel hen ongevoelig te maken voor pijn, wat zou kunnen begrepen worden uit het woord 'verdoven' (7).

SLACHTMASKERS, SCHIETTOESTELLEN

Allicht ten overvloede mag hierbij opgemerkt worden dat het ontstaan en voortbestaan van de verschillende methoden, met of zonder vooraf toegebracht hersenletsel, niets te maken hebben met meer of minder diervriendelijkheid. Vanaf de tweede helft van de 19de eeuw kwam er echter een beweging op gang die inging tegen brutaliteit en wreedheid bij het doden van dieren voor consumptie. Tegen het einde van die eeuw werden bij ons op instigatie van de toenmalige vereniging voor dierenbescherming, een uitvinding van de Parijzenaar Bruneau het slachtmasker geïntroduceerd. In het Gentse slachthuis werden ze in 1875 door een gemeenteraadsbesluit verplicht gemaakt, behoudens uitzonderingen bepaald door de slachthuisdirecteur (8). Die bevatten een pen (bout of pen): een metalen bout die als doorslag (drevel) fungeert. Een hamerslag drijft de pen (Figuur 2 en 3) in de schedelholte. De hersenen worden rechtstreeks beschadigd, waardoor het dier ogenblikkelijk bewusteloos neervalt. De toestellen van de eerste generatie waren voorzien van lederen zijstukken om de ogen

te bedekken. Later werden de toestellen geperfectioneerd door de pen te laten aandrijven door middel van een cartouche (slaghoedje, ontsteking) (Figuur 4). De zijstukken verdwenen, maar de term schietmasker bleef in gebruik.

De bewusteloze dieren blijven enige tijd stuiptrekken. Dit zijn bewegingen gecommandeerd door het evolutionair meest primitieve deel van de hersenen, de zogenaamde reptielenhersenen van zoogdieren. Ze worden geëlimineerd door een 'spinalisatiepen' ('riet') doorheen het schietgat tot in de kleine hersenen en het verlengde merg te steken (verboden sinds de BSE-epidemie).

De penmethode is veel gemakkelijker en vooral veel zekerder dan de directe hamerslag wanneer ze toegepast wordt door geoefende 'schieters'. Iedere schedel verschilt! Schedels van op die manier 'geslagen' dieren zijn goed herkenbaar (Figuur 5). Hoewel het misschien niet zo evident is voor wie het nooit zag gebeuren, is de pijn bij de inductie van bewustzijnsverlies minimaal, vanwege het geringe aantal pijnreceptoren dat geactiveerd kan worden. Met elektrische bedwelming of CO_2 -toediening kan een gelijkaardig bewustzijnsverlies bereikt worden. Daarmee werd in de jaren dertig van de vorige eeuw al veel geëxperimenteerd, vooral om de techniek van het joodse rituele slachten te verbeteren. Enkel de hersenen zouden beïnvloed worden en het dier zou 'rein' blijven. Dit werd echter door het rabbinaat nooit aanvaard. De elektrische methode brak pas na W.O. II goed door. Hoewel oorspronkelijk uitgedacht voor runderen, wordt deze methode vooral bij varkens en ander kleinvee toegepast (9). Voor beide methoden wordt in het Engels de term 'stunning' gebruikt.

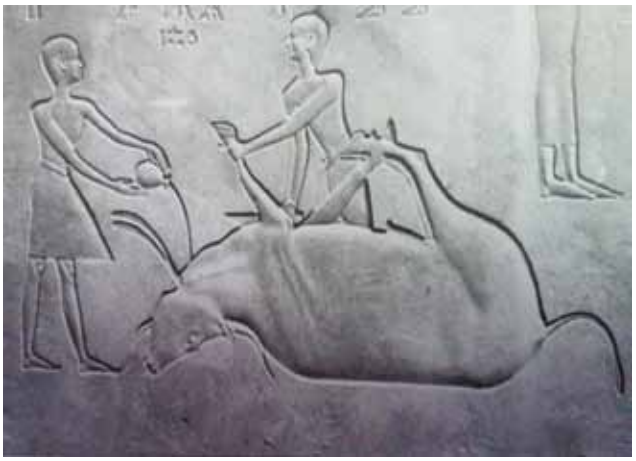
Van angst kan in de weinige seconden die nog volgen, geen sprake zijn. Wat daaraan voorafgaat blijft echter problematisch. Denk aan de enorme schrik van een schaap dat van een groep afgezonderd wordt, aan de angstsignalen (geluiden, geur, bewegingen, defecatie) van lotgenoten naar de slachtvloer geleid. Sommigen stellen dat niet zozeer het al of niet bedwelmen van belang is, als wel het goed uitvoeren van, hetzij het bedwelmen, hetzij de halssnede. Met beide kan er heel wat mislopen (10). Bij pen en elektriciteit duurt het een fractie van een seconde, bij ritueel slachten circa 9 seconden bij het schaap en 45 seconden bij het rund. Vanuit een toegenomen aandacht voor dierenwelzijn vinden veel mensen dit in een moderne samenleving niet langer acceptabel. Politiek speelt hierin een rol. Denk aan islamofobie. Men vergeet bijvoorbeeld al dan niet moedwillig dat bij CO_2-bedwelming van varkens het ook 45 seconden duurt voordat de dood intreedt.

De hierboven kort aangehaalde manieren van bedwelmen zijn minder brutaal, maar in feite niet wezenlijk verschillend van het oude knock-out slaan. Nog in 1947 werd de geschiedenis van het slachten in een Amerikaans standaardwerk als volgt (letterlijk) lapidair samengevat: 'There have been few changes or improvements in slaughtering since the first caveman (holbewoner) knocked down a wild bull with a rock or a stone axe and then stuck and skinned it with his flint knife (steenscherfmes)' (11).

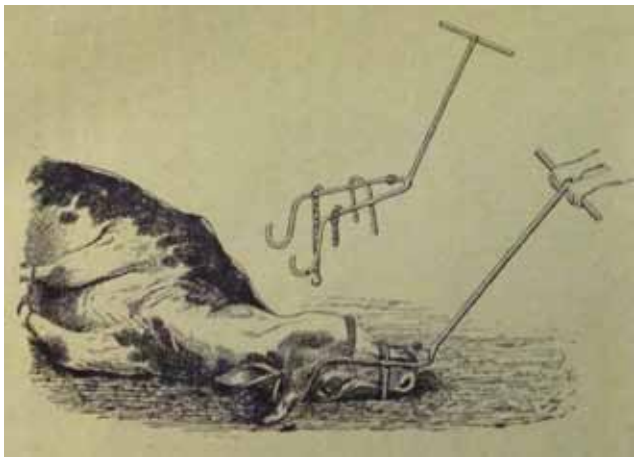
Bij het einde van dit tekstgedeelte moet nog iets gezegd worden over de uitdrukking 'humaan slachten' (humane slaughter). In het Engels is de term al meer dan een eeuw in gebruik, maar in het Nederlands is hij niet zomaar acceptabel. De term is dubieus. Duidt hij op een goedaardige, of minstens niet-wreedaardige manier van handelen? In tegenstelling tot wat het woord suggereert, is dit allesbehalve een universeel kenmerk van de menselijke soort. Of duidt dit op manieren van slachten die de gevoelens van mensen ontzien? De stadsmens anno 2016 wil het allerbeste voor zijn 'pets', maar wil niet geconfronteerd worden met het slachten, wil ook geen kadavers of karkassen zien, enkel netjes verpakte lapjes en hamburgers of onherkenbaar verwerkt vlees in klaargemaakte hapjes (12).

Figuur 5. Runderschedel met schietgat (MuMo, museumcollectie vakgroep Morfologie, Faculteit Diergeneeskunde, UGent, Merelbeke).

Figuur 6. 'Kollesteek' (uit: 'Moderne beenhouwerij en charcuterie', 1965).



Figuur 7. Het slachten van een os als offer. Sarcofaag van prinses Ashwit, Thebe, elfde Dynastie (2134 – 1976 vr Christus).



Figuur 8. Illustratie van immobilisatie destijds in gebruik voor het joodse rituele slachten (bron: zie Figuur 2).

De term humaan is hoe dan ook antropocentrisch: niet wat het dier ondergaat primeert, maar wat de mens ervaart. Toch wordt de uitdrukking humaan slachten soms gebruikt, wellicht omdat 'diervriendelijk slachten' een contradictio in terminis lijkt.

'NEKKEN' - NEKSTEEK

Het bewusteloos slaan en vervolgens doodsteken was niet overal traditie. Verre van. In grote delen van de wereld, in Spanje en haar Spaanssprekende ex-kolonies, in sommige Aziatische en Afrikaanse landen en ook in Rusland was nog een heel andere methode in gebruik. Daar stak men de verbinding door van het ruggenmerg met de hersenen via een holte tussen de schedel en de eerste halswervel, met een soort harpoen (Spaans: 'puntilla', of 'punctilla', in het Nederlands 'kollesteek' (kol: nek) (Figuur 6). Wanneer goed uitgevoerd, valt het dier ogenblikkelijk bewusteloos neer. Deze methode (Nederlands: 'nekken', Frans 'énucage') is bekend uit de stierengevechten in Spanje. Slachthuis is 'matadoro' of 'degolladero' in het Spaans. Het eerste woord is afgeleid van 'matar' (doden), het tweede duidt op 'nekken'('degollar' uit het Latijn 'decollare'). Bij kleinvee (pluimvee, duiven, konijnen) worden vergelijkbare vormen van 'cervicale dislocatie' (nekslag) algemeen toegepast, al of niet na bedwelming.

JOODS RITUEEL SLACHTEN, TABOE OP BLOED

In de hier beschreven manieren van slachten met het buiten bewustzijn brengen van het dier voorafgaand aan het dood steken, lag eeuwenlang het grote verschil tussen het in onze streken gebruikelijke slachten en het joodse rituele slachten (shechita, ook schächten genoemd). In het door het rabbinaat in detail voorgeschreven en streng gecontroleerde slachtritueel mag het zenuwstelsel niet geraakt worden. Bij de halssnede moet alles (huid, spieren, slokdarm, luchtpijp, halsslagaders) met een vlijmscherp mes in één of twee halen doorgesneden worden, zonder evenwel de kop van het lichaam te scheiden. Dit gebeurt op het op de rug liggend dier. Deze manier van doden was wellicht algemeen in het Midden-Oosten. Er bleven afbeeldingen van bewaard uit het faraonische Egypte (Figuur 7). De ruggengraat met het ruggenmerg moet intact blijven. Schade daaraan zou het uitbloeden nadelig beïnvloeden. Om dit goed te doen, moet het dier vooral stevig geïmmobiliseerd zijn. Oorspronkelijk gebeurde dat met een kluister (Figuur 8), nu met een draaibare kooi ('ren' of 'ben', Engels: 'pen'). Ook is de extreme angst voor de aanwezigheid van bloed allesoverheersend in de verdere behandeling van het vlees door de 'poorser', die zorgvuldig bloedvaten opzoekt en verwijdert, en in het verwerpen als therifa (treife, niet koosjer) van sommige lichaamsdelen, waaronder de culinair meest waardevolle: de achterkwartieren (13).

Wel neemt men alle mogelijke voorzorgen om doodsangst te vermijden: het slachtdier wordt afgezonderd, het mag geen getuige zijn van het slachten van een ander dier. Men is er in die kringen van overtuigd dat het shechita doden minder pijnlijk en (voor het dier) minder angstaanjagend is dan de methoden die het slachtdier buiten bewustzijn brengen voorafgaand aan het eigenlijke doden. Het is een mening die wellicht enkel onder orthodoxen gangbaar is en die tegengesproken wordt door vergelijkende metingen van de hersenactiviteit (14). Het complexe immobiliseren wekt hoe dan ook meer angst op dan wat er nodig is voor de penmethode en voor het elektrisch bedwelmen (15). Het aantal pijnreceptoren in de halsstreek is bovendien veel groter dan op het voorhoofd. Wel werd aangetoond dat de shechita slachtmethode voor kippen, waarbij de dieren niet opgehangen worden, maar de halssnede krijgen direct na het uit de kratten halen, diervriendelijker is dan het conventionele Europese en halal doden.

Vanwaar deze strenge geboden en verboden? Eerder al werd gewezen op een zekere afkeer voor de slachtpraktijk die bij herdersvolken bestaat, in tegenstelling tot wat (uiteraard) bij de jagers het geval is. Het bijbelse taboe op bloed van slachtdieren houdt wellicht verband daarmee. Daar komt nog bij dat bloed aanzien werd als iets uiterst belangrijks, volgens de bijbel gelijk te stellen met de 'ziel', in de rooms-katholieke bijbelvertaling: 'levenskracht'. Deze geheimzinnige en ook vreesaanjagende substantie mocht in geen geval door mensen opgenomen worden via het voedsel. Zelfs contact met de huid moest vermeden worden. Het bijbelboek Leviticus dat gedetailleerde voedselvoorschriften aangeeft, herhaalt en expliciteert in uiterst strenge bewoordingen dit principe dat al in het eerste bijbelboek Genesis in essentie vervat zit (16). Vandaar ook dat de getuigen van Jehova bloedtransfusies weigeren. Ook de schrik voor menstruatiebloed bij sommige ultrareligieuze mannen kadert in deze sfeer. Rein en onrein zijn hierbij kernbegrippen (17).

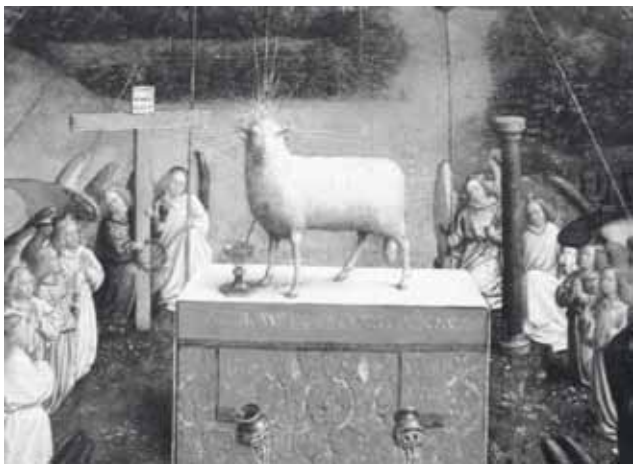
In de dominante, christelijke traditie bestonden en bestaan dergelijke geboden en verboden niet. Wel werden tijdens de middeleeuwen bijvoorbeeld in Zuid-Franse steden reglementen uitgevaardigd die streng verbod legden op de verkoop van 'viande sagatée' in de publieke vleeshuizen. Dat vlees was afkomstig van dieren gekeeld volgens de joodse ritus door de 'sagataire ', afgeleid van het Latijn 'sagitarius' (de sjoheet). Daarover was in de middeleeuwse steden soms heel wat te doen, en dit beslist niet omwille van diervriendelijkheid of medeleven, maar om vermeende kwalijke hoedanigheden van het aldus bekomen vlees (18). Schrik voor bezoedeling was algemeen. Stoffen waarvan aangenomen werd dat ze schadelijk waren, werden als 'besmettelijk' aanzien. Bij joden en christenen was de schrik wederzijds.

DHABIHA SLACHTEN VOOR DE PRODUCTIE VAN HALAL VLEES

In het joodse slachtritueel zit al het aspect offeren, wat het sterkst tot uiting komt in het verhaal van Abraham en in de paasmaaltijd (zie verder). Tot voor enkele decennia had men in onze streken geen weet van slachten volgens de islamitische voorschriften, waarin het offeren nog sterker doorleeft. Dit moet gebeuren volgens het slachtritueel (dhabiha) gebaseerd op koranvoorschriften voor de productie van halal (voor moslims toegelaten) vlees. Vier koranteksten hebben het over het slachten van dieren. We geven ze hier weer aan de hand van de Nederlandse vertaling van Kramers (19). In de tweede sura (spreek uit: soera, met de klemtoon op de oe) heet het: hij (God: Allah) heeft slechts verboden gemaakt het verdorvene, bloed, zwijnenvlees en wat geslacht is met een aanroep die niet tot Allah was, maar wie door nood gedreven is, zonder het te begeren en zonder te overtreden, voor hem is het geen zonde. In de vijfde sura: verboden is voor u het verstorvene en bloed en zwijnenvlees en wat niet geslacht is met een aanroep die niet tot Allah was, en het gewurgde, het dood geslagene (20), het afgestorte, het met de hoornen gespieste en waarvan wilde dieren gegeten hebben, behalve wat gij geslacht had, en wat geofferd is op het afgodsblok. In de zesde sura wordt gesteld dat verboden enkel gelden voor iets wat verstorven is en voor gestort bloed of zwijnenvlees, want dat is een gruwel, of iets verwerpelijks waarover een ander dan Allah is aangeroepen (tenzij in nood). De zestiende sura herhaalt nog eens: Hij heeft voor u verboden verklaard, slechts het verstorvene, en bloed en zwijnenvlees en dat waarover een ander dan Allah is aanroepen (behalve noodgedwongen). De nadruk ligt telkens op het aspect offeren, en wel enkel en uitsluitend aan Allah.

In sura 29, analoog aan het evangelie volgens Mattheus (21), komt een reële bezorgdheid om dierenwelzijn tot uiting: 'en hoe menig dier is er dat niet zelf zijn onderhoud bijbrengt, God bezorgt zijn onderhoud en ook het uwe'. De strafste bewoordingen hieromtrent treffen we aan in het bijbelboek Prediker (hoofdstuk 3, vers 19). We citeren (22). 'Mensen zijn niet meer waard dan dieren, want ook mensen leven maar kort. Mensen en dieren gaan op dezelfde manier dood. Ze zijn uit aarde gemaakt, en ze worden weer aarde. Gaat de laatste adem van de mensen misschien naar boven? En die van de dieren naar beneden? Dat weet niemand'.

Verdedigers van het niet bedwelmd slachten om religieuze redenen stellen dat een levenslange goede behandeling van de huisdieren veel belangrijker is dan de korte doodstrijd. Het is een stelling die ook verwoord wordt in meer neutrale werken die het gedrag van mensen tegenover dieren beschrijven (23). Dieren die als vee kunnen gehouden worden en last-



Figuur 9. Detail uit het Lam Godsretabel in de Gentse Sint-Baafskathedraal. In deze theologisch goed onderbouwde voorstelling wordt het lam weergegeven als een flink uit de kluiten gewassen volwassen dier, wellicht een eenjarige ram zoals het voorschrift in de Thora voor het pesah (paas)maal vereist.

dieren zijn overigens geschapen tot gerief van de mens (in sura 16), een opvatting die ook vervat zit in het welbekende bijbelverhaal van Noah.

De koranvoorschriften voor wat niet toegelaten dieren betreft, houden een uitdrukkelijk aangeduide inperking in van de complexe voedselverboden bij Joden en wellicht nog andere stammen uit het antieke Midden-Oosten. Zoals in de joodse voorschriften is varkensvlees niet toegelaten (haram), maar enkel dat. Ook bijna woordelijk verwant is het verbod op het eten van bloed, van vlees van gewurgde dieren of vlees dat op een of andere manier zou kunnen bedorven zijn: vlees van een dood gevonden dier, al of niet aangevreten door wilde dieren.

De koranregels voor het eigenlijke slachten kunnen als volgt worden samengevat: alleen vlees van een (toegelaten) dier waarbij tijdens het doden Allah aanroepen werd, is voor consumptie door gelovigen toegelaten (halal). Dit zijn de essentiële voorschriften: deze van de koran! Hoe zit het dan met de praktijk? De koranteksten zelf gaan niet in op de eigenlijke slachttechniek. In de plaatselijke moslimtradities gelden wel tal van afgeleide gebruiken. De koranregels werden verder geïnterpreteerd in afgeleide voorschriften en gewoonten, die resulteerden in het 'dhabiha' slachten. Die kent twee varianten. Bij runderen worden ofwel de halsslagaders aan de basis van de nek doorgesneden, ofwel de keelstreek. Bij kleinvee en gevogelte wordt enkel de tweede manier toegepast. Het offergebaar bestaat in het uitspreken van enkele gebedswoorden door een moslim en het richten van het dier naar Mekka. Het dier mag niet doodgeslagen worden en het moet goed uitbloeden. Het goed laten uitbloeden is overigens onderdeel van gelijk welke goede slachttechniek. Vlees van een niet goed uitgebloed slachtdier bewaart minder lang. Gebruik makend van objectieve wetenschappelijke methoden ("packed cell volume" en percentage bloedverlies) kon geen verschil in uitbloeding aangetoond worden tussen slachten zonder bedwelming (zoals toegepast door moslims) en met de pen of met elektrisch bedwelmen (24)

In het Franse encyclopedische werk over het slachten en het slachthuiswezen van de Loverdo, dat uit 1906 dateert - lang voordat het onderwerp controversieel werd - staan de moslimgebruiken, zoals door de auteur geobserveerd in Noord-Afrika, als volgt beschreven: (vertaling) 'Moslims is het verboden vlees te eten van dieren die niet door geloofsgenoten geslacht werden en waarvan het hoofd op het moment van het slachten niet naar het oosten gekeerd is. Op dat punt zijn ze categoriek. Ze weigeren zelfs het heerlijkste vlees waarvan ze de origine niet kennen met het voorwendsel dat het slecht uitgebloed is' (25). In latere tijd is er blijkbaar een verstrenging opgetreden in de opvattingen in dezelfde regio.

De koranteksten geven dus geen richtlijn aan voor het al of niet bedwelmen..Wel kan het verbod op doodslaan in sura 5 in de richting van een verbod op het induceren van bewustzijnsverlies (verkeerd) geïnterpreteerd worden. Het al of niet dood zijn na het bedwelmen met de penmethode kan een inhoudsloos discussiepunt worden: deze methode resulteert in de dood, maar pas na ettelijke (tot tien) minuten (zie noot 8). Het kelen of het 'steken' van de aorta gebeurt echter vrijwel onmiddellijk na de penslag bij het nog levende dier. Het reversibele elektrisch bedwelmen wordt door sommige moslimautoriteiten aanvaard, door anderen niet (26). In tegenstelling tot wat meestal verkondigd wordt, en wat de meeste moslims zelf geloven, kan halal vlees dus afkomstig zijn zowel van bedwelmd als van niet bedwelmd geslachte dieren (27). Dat eerste is met name het geval met vlees uit Nieuw-Zeeland, wereldwijd het grootste exportland van lamsvlees.

De praktijk werd gecompliceerd door het willen voortzetten door migranten in Europa van wat in de arme dorpen van herkomst gebruikelijk was, met name het eigenhandig slachten in primitieve omstandigheden van een schaap voor het Offerfeest (waarover verder meer), in het slechtste geval door de ongeoefende familievader. De grootschalige productie van halal vlees, waarbij, net als in alle industriële slachterijen alles gericht is op snelheid, stelt de rituele praktijk overigens voor grote problemen.

SLACHTEN IS OFFEREN

De koranteksten laten zeer duidelijk blijken wat de essentie van het slachten door en voor moslims inhoudt: het offeren, meer bepaald enkel aan de éne ware God, Allah. Bovendien bevat de koran nog andere teksten die meer specifiek op het offeren gericht zijn. Die komen het beste tot uiting in het jaarlijkse, grote Offerfeest, waarin het offer van Abraham herdacht wordt. Iedere moslimfamilie die het financieel aankan, wordt geacht een schaap te (laten) slachten: te offeren of, als dit niet kan, een in waarde min of meer vergelijkbare aalmoes te geven. Het vlees wordt feestelijk geconsumeerd en verdeeld onder vrienden en buren en in het bijzonder ook onder de armen.

Om te beginnen moet gesteld worden dat dit sinds de prehistorie een wijd verspreid gebruik was. Getuigenissen in schrift en beeld uit het oude Egypte zijn bekend (28). Een opmerkelijke variant stamt uit Indië, waar de heilige koe het offerdier bij uitstek was (29). Het Brahmaanse offerritueel vervulde een rol in de complexe regeneratie (wedergeboorte) gedachte. In het Hebreeuwse pesah, de herdenking van de verlossing uit de Egyptische slavernij door toedoen van Mozes (de exodus), zit eveneens een aspect offeren (30). Volgens de bijbelse overlevering gaf Mozes zeer nauwkeurige instructies voor het slachten en feestelijk consumeren bij die gelegenheid van een gezonde schapenram of geitenbok, één jaar oud, weerom niet toevallig een mannelijk dier.

Vanwaar dat offeren? En waarom dieren offeren? Hoewel massaal slachten nu alledaags is, louter utilitair, en in niets gelijkt op het brengen van een offer,

heeft het vluchtige offerritueel bij het dhabiha slachten wel degelijk een oeroude achtergrond van offergave. In de meeste culturen werden offers gebracht om de hogere machten gunstig te stemmen of om aan hun vermeende wensen te voldoen. In zijn meest extreme gedaante vormt het slachtdier als offer een surrogaat voor het mensenoffer. Denk aan het verhaal van Abraham die op het punt stond zijn enige zoon aan God aan te bieden (te offreren: offeren) en dat op de meest drastische manier die mogelijk is: door hem te doden. God verhindert dat en stelt Abraham in de gelegenheid een bokje - niet toevallig een bokje - te nemen als offer. Deze opmerkelijke episode wordt uitgelegd als een herinnering aan nog oudere praktijken waarin koning-priesters (priester-koningen) in tijden van hoge nood geacht waren zichzelf te (laten) offeren (31). Het in oorsprong neutrale woord offeren, Latijns van oorsprong (‘dragen naar’), kreeg dus een belangrijke religieuze betekenis (32).

SLACHTRITUELEN EN VOEDSELTABOES OPGEHEVEN

Het paasfeest ontleende zijn naam aan pesah. Het laatste avondmaal van Jezus met zijn leerlingen (apostelen) was in feite een pesahmaal. De symboliek van dit christelijk gebeuren en de verwevenheid met de Hebreeuwse tradities reiken nog veel verder, en wel in de essentie ervan: het offer van Jezus, de mensenzoon van God, die door zijn dood verlossing bracht aan de mensheid (33). Bij de kruisiging werd op dramatische manier het offer van Abraham herhaald, echter zonder dat er een dier aan te pas kwam en, net als bij Abraham, met een happy end: de verrijzenis. Jezus zelf is het lam, het ‘Lam Gods (Agnus Dei) dat wegdraagt de zonden van de wereld’. We zien het wondermooi voorgesteld op het Gentse Lam Godsretabel van de van Eycks (Figuur 9). In de katholieke traditie wordt dit dagelijks herdacht in het misoffer.

In de context van het hier behandelde onderwerp is dit uiterst belangrijk. Daarin zijn immers de theologische uitleg en rechtvaardiging te vinden van het verdwijnen van voedseltaboes en slachtrituelen in het christendom: Jezus had door zijn offerdood de strenge joodse voorschriften overbodig gemaakt. Het was een inzicht dat al kort na de dood van Jezus door de verromeinste apostel Paulus ingezet was, toen hij de evangelische boodschap, erfplichtig aan de Hebraïsche traditie, naar niet-Joden bracht (34). In de eerste eeuwen van het christendom bleven sommige verboden, zoals dat op het eten van varkensvlees, nog doorleven, maar ze verdwenen tenslotte totaal. Rond vlees en zelfs zuivel en eieren bleef wel een zekere taboesfeer hangen in diepchristelijke kringen, zoals bij de monniken. Leken konden het stellen met het ‘derven’ van vlees op sommige dagen. Dat was de verplichte vastenpraktijk (35).

Het aspect ‘offeren’ is bij het conventionele slachten totaal verdwenen. Merkwaardig genoeg bleef dat nog voortleven in het wetenschappelijk onderzoek. Proefdieren worden niet gedood, maar geofferd, wat blijkt uit het overvloedig gebruik in de biomedische wetenschappelijke literatuur van de term ‘to sacrify’. Zo heet het, bijvoorbeeld, dat ‘y mice were sacrified in experiment x’. Dit Engelse woord betekent letterlijk ‘heiligen’, maar is synoniem met ‘offeren’. Een levend wezen wordt immers heilig gemaakt doordat het geofferd wordt, of zichzelf offert: het ‘sacrificium’ (36). Proefdieren worden opgeofferd voor ons welzijn.

EPILOOG

Ook buiten de dhabiha slachtpraktijk leven we nu nog bestendig met het idee van offeren, althans in courante uitdrukkingen zoals ‘zijn leven offeren’, ‘zich opofferen’ en in het woord ‘slachtoffer’. Dat laatste werd in het Nederlands voor het eerst neergeschreven halverwege de 16de eeuw en wel in de christelijke betekenis van ‘geslacht om te offeren’. Het was de tegenhanger van het ‘brandoffer’: een waardevol voorwerp dat verbrand wordt om te offeren aan een godheid, een gebruik waaraan de offerkaarsen in katholieke en andere heiligdommen nog herinneren. Bedenk daarbij dat waskaarsen – uit bijenwas – vroeger uiterst kostbaar waren. Het Franse en Engelse ‘victim(e)’ werd ontleend aan het Latijnse ‘victima’: levend schepsel als offer aangeboden aan de goden. Pas vanaf halverwege de jaren 1700 werd ‘slachtoffer’ in figuurlijke betekenis gebruikt (37). Het courante gebruik van dit woord waarin zowel slachten als offeren zit, toont aan hoever we verwijderd zijn van de oorspronkelijke betekenis. De zoveel of zoveel slachtoffers van natuurrampen, verkeersongevallen en gewapende conflicten, waarover dagelijks bericht wordt, hebben niets te maken met slachten en veel minder nog met offeren.

Slachten en ‘slaan’ was overigens nooit alleen maar iets dat beperkt bleef tot het doden van dieren voor menselijke consumptie. Denk aan de welluidende termen slagveld, veldslag, slag leveren, slagorde, slagkracht, slachting en verslaan, woorden en uitdrukkingen die teruggaan tot de tijd dat onze stoere voorouders mekaar de kop insloegen met knotsen, exacter uitgedrukt: mekaar neersloegen. Ook in ‘nederlaag’ en ‘het onderspit delven’ zit dat vervat. De slagkracht van de wapens is nu wel een miljoenvoud groter, maar de hersenen van de mensen die ze maken en commanderen, zijn in de heel korte tijdspanne waarin deze ongelooflijke verandering plaats greep, evolutionair niet veranderd. Ons taalgebruik en de dagelijkse nieuwsberichten verraden het.

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la plus succulente, s'ils en ignorant la provenance, sous pretexte qu'elle a été mal saignée' (de Loverdo J. 1906, vol. 1, 886 en vol. 2, 161-162); Brissebarre, A.M. (1999). Sacrifice et abattage rituels musulmans: comportements et représentations en milieu urbain en France et au Maroc. In: Quedraogo A.P., Le Neindre, P. (eds.) *L'homme et l'animal: un débat de société*, INRA, Parijs, 189-206.
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Postpartum uterine diseases in dairy cows: a review with emphasis on subclinical endometritis

Baarmoederaandoeningen na het afkalven bij melkkoeien: een overzicht met nadruk op subklinische endometritis

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ABSTRACT

In this review, updated and precise definitions of the most common postpartum uterine diseases in dairy cows are provided. An aberrant uterine environment at inappropriate stages of the reproductive cycle inflicts damage to gametes and zygotes, impairing the reproductive performance of dairy cows. This involves major economic losses for the milk production unit. Consequently, an accurate diagnosis of postpartum uterine diseases is indispensable for practitioners to set up a prompt and efficient treatment. This review furthermore emphasizes on the new perspectives regarding diagnosis and treatment of subclinical endometritis, a highly prevalent uterine disease that is often overlooked by practitioners while causing major reproductive problems. Based on a more profound clinical understanding of the postpartum uterine disease complex, practitioners will be able to better use the available diagnostic tools and therefore apply a more efficient therapeutic approach.

SAMENVATTING

In dit artikel wordt een geüpdatet overzicht gegeven van de definities van de meest voorkomende baarmoederproblemen na het afkalven bij melkkoeien. Een afwijkend uterien milieu op de meest precaire momenten van de voortplantingscyclus veroorzaakt immers schade aan de gameten en het jonge embryo met finaal nefaste gevolgen voor het voortplantingsvermogen van de koeien. Dit laatste zorgt voor beduidende economische verliezen op veel moderne melkveebedrijven. Vandaar dat het voor de practicus van groot belang is om tot een accurate diagnose te komen teneinde een gerichte en efficiënte behandeling te kunnen instellen. Bovendien wordt in dit artikel bijzondere aandacht besteed aan subklinische endometritis. Deze aandoening is bij practici minder goed bekend terwijl ze bij een relatief groot aantal dieren voorkomt op het moment van inseminatie. Via het aanreiken van diepgaandere kennis omtrent het complex van postpartum baarmoederproblemen, hopen de auteurs met dit artikel de practicus te wijzen op de voorhanden zijnde mogelijkheden om tot een accurate diagnose en dus ook meer efficiënte behandeling te komen.

INTRODUCTION

Postpartum uterine disease is the leading cause of reproductive inefficiency in dairy cattle (Barlund et al., 2008). Dairy cattle farmed in intensive systems, commonly acquire microbial contamination of the uterus during parturition (Sheldon et al., 2008). Almost all dairy cows (80 to 100%) experience bacterial intrauterine contamination immediately after calving (Herath et al., 2006; Sheldon et al., 2009). Due to this fact, as well as due to the substantially required repair of the endometrium following parturition, uterine inflammation is a normal and necessary component of the postpartum uterine involution process (LeBlanc, 2014). However, in a proportion of the postpartum cows, the inflammation runs out of control and leads to uterine disease (Chapwanya et al., 2010).

An elevated postpartum uterine disease incidence contributes to reduced fertility interfering with the main goal of efficient reproductive management: to have cows pregnant at a biologically optimal and economically profitable time after parturition (Sheldon

Table 1. General overview of the main characteristics of the most common postpartum uterine diseases.

Postpartum uterine disease	Definition	Days after calving and reported incidence	Treatment
Retention of fetal membranes	Failure to expulse the placenta between 12 to 24 h after parturition	24 hours after parturition incidence: 4 to 12%	Strong traction is discouraged Local antibiotics?
Metritis (puerperal and toxic)	Enlarged, atonic uterus Fetid, watery red-brown discharge Signs of systemic illness (fever >39.5°C, decreased milk yield, signs of toxemia)	Within 21 days after calving Usually at the end of the first week after calving incidence: 5 to 15%	Local Antibiotics? In case cows become febrile, systemic antibiotics for at least 3 consecutive days Supportive therapy if required
Clinical endometritis and/or purulent vaginal discharge	Local inflammation of the endometrium (clinical endometritis) Presence of purulent or mucopurulent material in the vagina (PVD) Absence of systemic symptoms (fever)	≥ 21 days after parturition incidence: 20 to 30%	Intrauterine antibiotics (cephapirin) ≥26 after parturition Benefit of PGF2α is not clear
Pyometra	Presence of purulent material in the uterine lumen Corpus luteum present Cervix often closed	Depending on the days post calving after first ovulation incidence: 1 to 2%	Two doses of PGF2α with an interval of 11 to 14 days between applications
Cytological endometritis	Abnormal presence of PMNs in endometrial cytology samples Absence of clinical sign of purulent vaginal discharge	Diagnosed between 21 to 64 days after calving or during AI incidence: 9 to 76% most commonly between 25 to 35%	Intrauterine antibiotics (cephapirin) or PGF2α (both under discussion) Therapeutic flushing of the uterus?

et al., 2006; Gilbert, 2016). An inflammatory milieu at inappropriate stages of the estrus cycle interferes with fertility by creating suboptimal conditions for sperm transportation and storage, oocyte maturation and ovulation, zygote development, implantation, and embryonic and fetal growth (Gilbert, 2011). In this context, subclinical endometritis (SCE) is a highly prevalent disease that runs without clinical symptoms significantly impairing the fertility of dairy cows (Sheldon et al., 2006; Barlund et al., 2008). However, standardization of SCE diagnosis is not fully established yet (Pascottini et al., 2015). Therefore, an updated and clear definition of the postpartum uterine diseases may help practitioners in order to properly examine and accurately diagnose postpartum cows to set up an efficient treatment prior to the start of the breeding period.

Thus, the aim of this paper was to review the postpartum uterine disease complex, such as retention of fetal membranes (RFM), clinical metritis (CM), clinical endometritis (CE) and/or purulent vaginal discharge (PVD), and pyometra; with particular emphasis on SCE (Table 1).

RETENTION OF FETAL MEMBRANES

Retention of fetal membranes or retained placenta is the failure to expulse the placenta between 12 to 24 hours after parturition (Paisley et al., 1986; Fourichon et al., 2000; Drillich et al., 2003). If RFM occurs, the membranes are retained in the uterine lumen for an average of seven days (Eiler, 1997), enhancing bacterial contamination and delaying uterine involution (Laven and Peters, 1996). The incidence of RFM ranges from 4 to 12 % (Drillich et al., 2003), with a median incidence rate of 8.6% (Kelton et al., 1998). Predisposing factors are twins, dystocia, stillborn calf, abnormal length of gestation, induced parturition, abortion, nutritional imbalance, fetotomy, cesarean section, increasing age and seasonal effects (Laven and Peters, 1996; Beagley et al., 2010). Retained placenta has no direct impact on the milk production, reproduction or culling if the condition does not evolve to CM, CE, or SCE (Dubuc, 2011). The increased risk of the previously mentioned diseases constitutes the main reason of the economic importance of RFM. A variety of methods has been used to treat RFM; nevertheless, the topic is still controversial. Manual removal,

local antibiotics and ecbolic drugs are commonly used treatments, although current evidence does not support their use (Laven and Peters, 1996; Stevens and Dinsmore, 1997; Gilbert, 2016). Intrauterine infusion of oxytetracycline is a common treatment among practitioners. It may reduce the incidence of subsequent fever (Stevens et al., 1995). However, it has no effect on the subsequent reproductive performance (Goshen and Shpigel, 2006), and is associated with detectable milk residues, which can persist up to 144 hours (Dinsmore et al., 1996). Moreover, the local antibiotic therapy (especially with tetracyclines) may prolong the placental retention due to its ability to inhibit matrix metalloproteinases (Gilbert, 2016). In case cows become febrile, systemic antibiotics (ceftiofur) appear to be beneficial in reducing disease and aiding in the return to normal reproductive function (Beagley et al., 2010). However, in many, especially European countries, the systemic use of broad spectrum antibiotics is currently severely under pressure because of the potential association with the increase of antibiotic resistance.

CLINICAL METRITIS

Clinical metritis is characterized by an enlarged uterus and a watery, red-brown fluid to viscous, off-white purulent uterine discharge, which often has fetid odor, occurring within 21 days postpartum (Sheldon et al., 2006). The diagnosis of CM is made on the basis of clinical signs of fetid uterine discharge and/or systemic illness. The severity of the disease has been categorized according to the health signs of the animal in grades 1, 2 and 3 (Sheldon et al., 2009). Clinical metritis grade 1 is characterized by an abnormally enlarged uterus and uterine discharge without systemic signs of illness (Sheldon et al., 2006). Clinical metritis grade 2 (or puerperal metritis) refers to animals that suffer from additional signs of systemic illness, such decreased milk production, dullness, and fever > 39.5° C (Sheldon et al., 2006). Clinical metritis grade 3 (or toxic metritis) includes animals with clinical signs of toxemia, such as inappetence, cold extremities, depression, and/or collapse (Sheldon et al., 2009). The incidence of CM is between 15 to 20%, but it can be much higher in some herds (Gilbert, 2016). Risk factors for any degree of CM are often associated with RFM, dystocia, stillbirth or twins. Clinical metritis usually occurs at the end of the first week after calving, being less common after the second week postpartum (Markusfeld, 1984; Drillich et al., 2001). The impact of metritis on the milk production and reproduction is controversial (Dubuc, 2011). When reported as detrimental, the impact on milk production is between 2 and 13 kg of milk per day during a period of 2 to 20 weeks (Overton and Fetrow, 2008; Wittrock et al., 2009; Giuliodori et al., 2013). Giuliodori et al. (2013) proposed that puerperal CM is associated with an impaired early pregnancy rate and an extended calving to conception interval, while in other studies no link was found between CM and a decline in reproductive capacity (Dubuc, 2011). A common treatment for CM (puerperal/toxic) is the intrauterine infusion of antibiotics. However, the efficacy of a local antibiotic treatment is a controversial issue (Drillich et al., 2001). Nowadays, the use of systemic antibiotics (ceftiofur for three consecutive days) in cows with an abnormal vaginal discharge at days 4 to 6 after calving and a rectal temperature ≥ 39.5°C has gained popularity and is the most used treatment for puerperal/toxic metritis (CM grade 2 and 3), although this treatment has become controversial in terms of prudent use of antibiotics (Drillich et al., 2001; Zhou et al., 2001). In a recent, randomized clinical trial, the efficacy of the initial use of ketoprofen versus ceftiofur was studied in cows with CM to reduce the usage of antibiotics (Pohl et al., 2016). However, no beneficial effects were found when cows were initially treated with ketoprofen only; finally more doses of medical applications had to be expected (Pohl et al., 2016).

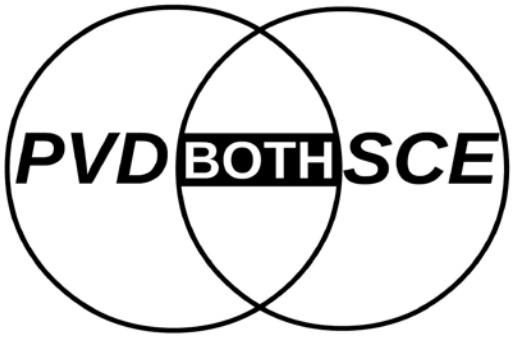

Figure 1. Cows may develop different uterine disease statuses: purulent vaginal discharge (PVD; cervicitis/vaginitis) only, subclinical endometritis (subclinical endometritis, SCE) only, or both PVD and SCE (clinical endometritis) (Adapted from Dubuc et al., 2010).

CLINICAL ENDOMETRITIS AND/OR PURULENT VAGINAL DISCHARGE

Clinical endometritis is basically referring to a local inflammation of the endometrium, characterized by the presence of purulent or mucopurulent (> 50% pus) material in the vagina ≥ 21 days postpartum originating from the uterus, not accompanied by systemic illness (Sheldon et al., 2006; Dubuc et al., 2010). It affects around 20% of dairy cows between 21 to 40 days postpartum (LeBlanc et al., 2002a). Usually, CE is diagnosed by means of a vaginoscope, a gloved hand or the metri-check (McDougall et al., 2007; Pleticha et al., 2009). However, it has become clear that the presence of abnormal vaginal exudate may not precisely be related to endometrial inflam-

mation. Endometritis requires endometrial cytology or biopsy (or ultrasound examination) for a convincing diagnosis (Gilbert, 2016). The presence of vaginal exudate nowadays is referred to as 'purulent vaginal discharge' (PVD). It is generally assumed that PVD is the result of endometritis, cervicitis/vaginitis or the combination of both (Dubuc et al., 2010; Deguillaume et al., 2012) (Figure 1). The prevalence of endometritis alone is around 13%, cervicitis only 11%, while 32% of the cows suffer from both conditions (Deguillaume et al., 2012). The detrimental effects of endometritis and cervicitis/vaginitis on reproductive performance are additive (Dubuc et al., 2010). In general, cows affected with PVD need on average thirthy days more to become pregnant than unaffected cows (LeBlanc, 2008; Dubuc et al., 2010; Dubuc, 2011). Currently, several controversial reports on the efficiency of treatment protocols for CE/PVD are available in the literature. However, two main approaches are commonly used: parenteral injections of prostaglandins (PGF2α) and intrauterine antibiotics (Lefebvre and Stock, 2012). Prostaglandins have been reported to be slightly beneficial (Kasimanickam et al., 2006) or inefficient (Galvão et al., 2009a). Routine use of PGF2α after 30 days postpartum may be relevant, but there is lack of evidence to sustain its efficiency (Dubuc, 2011). The use of an intrauterine cephapirin application after 26 days postpartum has been proven to be useful for treating PVD (LeBlanc et al., 2002b; McDougall, 2003; Runciman et al., 2008; Dubuc, 2011; Lefebvre and Stock, 2012; Tison et al., 2016).

PYOMETRA

Pyometra is defined as the accumulation of purulent or mucopurulent material in the uterine lumen provoking a distension of the uterus, accompanied by the presence of an active corpus luteum (Sheldon et al., 2006). In pyometra, the cervix is often functionally closed, although its lumen is not always completely occluded, and some purulent material may discharge through the cervix, vagina or vulva (Sheldon et al., 2006). Based on a large field study (Busch and Kuhnke, 2000), pyometra showed to affect approximately 1.2 % of the investigated cows, and the affection was related to problems during the postpartum period in most of the cases (Opsomer et al., 2000). Generally, ovulation is delayed in cows with a pathologic uterine load (Sheldon et al., 2002), but in case the cows do ovulate during an ongoing uterine infection, pyometra may develop (Gilbert, 2016). The diagnosis of pyometra can be done by rectal palpation and/or ultrasound, echography being the preferred and most accurate method to diagnose the disease. Treatment is based on the injection of two doses of PGF2α an with an interval of 11 to 14 days between the applications, with a fair rate of healing (Gustafsson et al., 1976; de Kruif et al., 1977; Olson et al., 1986). The prognosis after PGF treatment is generally favorable, with a first service conception rate of approximately 30% and an expected pregnancy rate of 80% after three or four inseminations (de Kruif et al., 1977; Fazeli et al., 1980).

SUBCLINICAL ENDOMETRITIS

Subclinical endometritis plays a critical role in the modern dairy industry. It is highly prevalent, asymptomatic and has a profound detrimental effect on the reproductive performance (Sheldon et al., 2006; Barlund et al., 2008). Subclinical endometritis has been indeed one of the most important reproductive impairments in dairy cows studied in the last decade (Pascottini et al., 2015). It is defined as a superficial inflammation of the endometrium, evidenced by relative increase in the number of PMNs in the uterine lumen, without visible clinical signs, but significantly impairing fertility (Bondurant, 1999; Kasimanickam et al., 2004; Gilbert et al., 2005; Sheldon et al., 2006). As SCE cannot be detected by simple visual inspection, complementary examinations are necessary for its diagnosis, being histopathology, ultrasonography, leukocyte esterase strips (LES) and endometrial cytology.

Endometrial biopsy and histopathology

In the bovine and equine literature, histopathology is considered the gold standard to diagnose endometrial alterations because it allows to directly visualize both acute and chronic changes of the endometrium (Bonnett et al., 1991; Chapwanya et al., 2010; Pascottini et al., 2016b) (Figure 2). In mares, for many years endometrial biopsy sampling has been regarded as an integral part of breeding soundness evaluation (Van Camp, 1988; Snider et al., 2011). However, in cattle, endometrial biopsy is rarely used since it is considered to be time-consuming, expensive and potentially detrimental for further fertility (Miller et al., 1980; Etherington et al., 1988; Bonnett, 1989; Sheldon and Dobson, 2004; Sheldon et al., 2006).

In an attempt to facilitate the SCE diagnosis and substitute the use of endometrial biopsy, it has been compared with endometrial cytology in mares (Overbeck et al., 2011; Nielsen et al., 2012) and in cows (Meira Jr et al., 2012; Madoz et al., 2014; Bogado Pascottini et al., 2016; Pascottini et al., 2016b). In general, these studies demonstrate that the agreement between cytology and histopathology (as the gold standard) is moderate, with a low sensitivity but very high specificity (Pascottini et al., 2016b). Cytology methods underestimate the health status of the endometrium of dairy cows. Generally, cytologic diagnosis is based on the mere visualization of the PMNs' proportion. On the other hand, histological examination of the endometrium may also reveal chronic changes, such as periglandular fibrosis, angiosclerosis, endometrial gland atrophy or lymphoid aggregates (Sheldon et al., 2006; Snider et al., 2011). Indeed, after re-

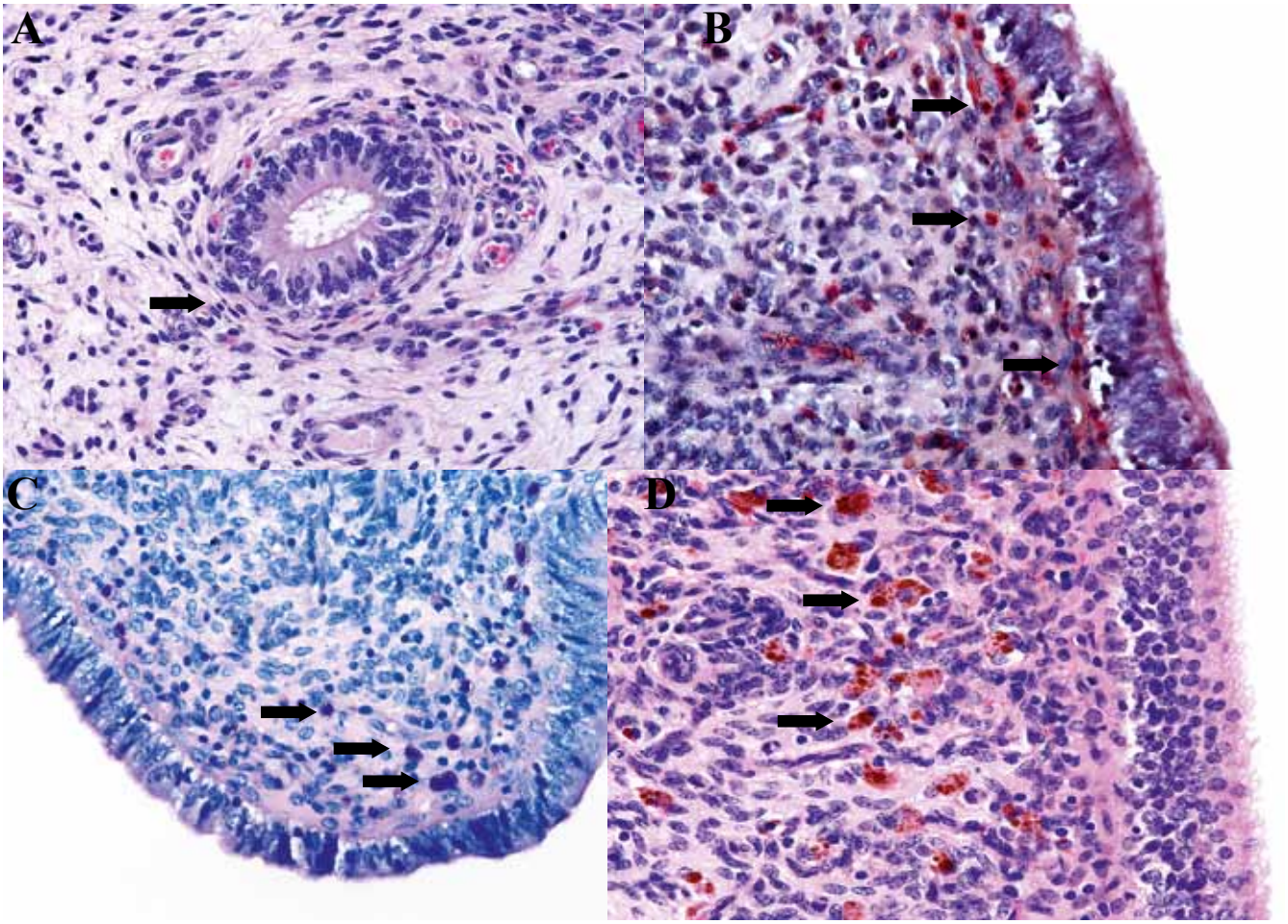

Figure 2. A. Mild periglandular fibrosis (arrow) in an endometrial histopathology sample stained with hematoxylin and eosin B. Polymorphonuclear cells stained in bright red (arrows) in an endometrial histopathology sample stained with Naphtol-AS-D-chloroacetate-esterase C. Mast cells (arrows) in an endometrial histopathology sample stained with Giemsa D. Hemosiderin (in macrophages; arrows) in an endometrial samples stained with hematoxylin and eosin.

covering from active endometritis (PMN infiltration), chronic alterations may persist (Sheldon et al., 2006), being left unnoticed when only applying cytological analysis (Bogado Pascottini et al., 2016). Therefore, a definitive conclusion about the uterine health can only be reached by an endometrial biopsy. However, to the best of the authors' knowledge, there are currently no papers available confirming the use of endometrial histopathology to accurately predict the reproductive capacity of the cow.

Subclinical endometritis and ultrasound

Nowadays, ultrasonography is considered to be an indispensable part of a routine clinical examination of the reproductive tract of dairy cows (Ginther, 2014). In this context, its potential to diagnose SCE has been demonstrated by multiple authors (Kasimanickam et al., 2004; Barlund et al., 2008; Meira Jr et al., 2012). The presence of fluid in the uterus between 20 to 47 days postpartum has shown to be associated with a significant reduction in the relative pregnancy rate in comparison to cows that appeared "clean" at examination (Kasimanickam et al., 2004; López-Helguera et al., 2012; Gobikrushanth et al., 2016; Šavc et al., 2016). However, the agreement between ultrasound and endometrial cytology is low, meaning that ultrasound and cytology measure two different representations of SCE. On the one hand, the clearance mechanism of the uterus (luminal fluid) and on the other, the cellular response of the inflamed uterus (PMNs in cytology slides) (Kasimanickam et al., 2004; Barlund et al., 2008). Another parameter used to diagnose SCE, is the endometrial thickness measured by ultrasound. However, this technique is underused since it can easily be influenced by the location of the probe on the uterine horn. Consequently, although ultrasonography is an easy and fast technique to apply in practice, it is considered to be not accurate when it is not accompanied with endometrial cytology (Barlund et al., 2008).

Leukocyte esterase colorimetric strips (LES)

LES has been used for rapid diagnosis of inflammation in fluids, such as urine, pleural, peritoneal and cerebrospinal fluid (Couto et al., 2013). Leukocyte esterase is a an enzyme produced by neutrophils, and

is therefore known to be indicative for inflammation. LES has been used as an indirect method to detect inflammation due to its reaction with the diazoniun salt released indoxil, which is oxidized, yielding a violet azo dye (Kutter et al., 1987). The intensity of the dye is related with the leukocyte counts. In order to create a "cow side" diagnostic method for SCE, LES were tested to assess their potential to diagnose SCE (Cheong et al., 2012; Couto et al., 2013; Denis-Robichaud and Dubuc, 2015a). Although results obtained by LES are positively correlated with endometrial cytology results (Cheong et al., 2012; Couto et al., 2013), they initially were not correlated with the odds of pregnancy (Couto et al., 2013). However, in a recent large study by Denis-Robichaud and Dubuc (2015a), a strong correlation was found between the LES results and the odds to become pregnant. Consequently, the authors considered the use of LES as a valid alternative for on-farm SCE diagnosis. However, to fully recommend the use of leukocyte esterase reagents as a cow-side diagnostic tool for SCE, this method needs further refinement (Cheong et al., 2012).

Endometrial cytology

Endometrial cytology is the most used technique to diagnose SCE in cattle in both field and research setups (Dubuc et al., 2010, de Boer et al., 2014). The assessment of the proportion of PMNs in cytology slides is the hallmark for SCE diagnosis, to the point that some authors refer bovine SCE to as "cytological endometritis" (CYTO) (de Boer et al., 2014) (Figure 3). Similarly to PVD, CYTO diagnostic criteria are established based on subsequent reproductive performances (Dubuc, 2011). In general, in comparison to their negative counterparts, cows affected by CYTO experience a detrimental effect regarding their reproductive capacity (Gilbert et al., 2005; Galvão et al., 2009a; Dubuc et al., 2010). Although there is no impact of CYTO on milk production (Dubuc, 2011), its importance is mainly based on the increased time to pregnancy (Gilbert et al., 2005) and the concomitant economic loss (Dubuc, 2011). Assuming that each "extra" open day costs to the farmer approximately 2 euro (Plaizier et al., 1997; De Vries, 2006), and that a CYTO positive cow has on average an increased time to pregnancy of 25 days (Gilbert et al., 2005; Dubuc et al., 2010), CYTO associated costs reach up to 50 euro per positive cow, plus the cost (material and service) of extra unsuccessful inseminations and eventual treatments. Moreover, CYTO has been shown to be a highly prevalent disease ranging on average from 20 to 30% of examined postpartum cows (Dubuc, 2011).

Endometrial cytology techniques in dairy cows: cytobrush and low volume lavage

Cytology is the science that evaluates the structure, chemistry and functionality of the cells (Grunze and Spriggs, 1983). Polymorphonuclear granulocytes represent the first and principal immunologic defense mechanism in the uterus (Sheldon and Dobson, 2004; Herath et al., 2006; Singh et al., 2008; Sheldon et al., 2009). Thus, an elevated number of PMNs in the uterine lumen indicates an inflammatory reaction of the endometrium (endometritis). Different techniques have been described to obtain endometrial samples for cytological examination in both mares and cows (Ball et al., 1988; Kasimanickam et al., 2004; Card, 2005; Gilbert et al., 2005; Barlund et al., 2008; Overbeck et al., 2011; Cocchia et al., 2012; Pascottini et al., 2015). In cows, cytobrush (CB) and low volume lavage (LVL) are the most used techniques to harvest endometrial surface scrapings. The CB consists of a small brush that is commonly used to sample cells from the vagina, cervix or endocervix for Papanicolaou testing in women (Venes, 2013). As such, CB use was first reported in women to collect cervical samples to diagnose malign tumors (Glenthøj et al., 1985). In 2004, for the first time, the CB was adapted to harvest endometrial samples in cows (Kasimanickam et

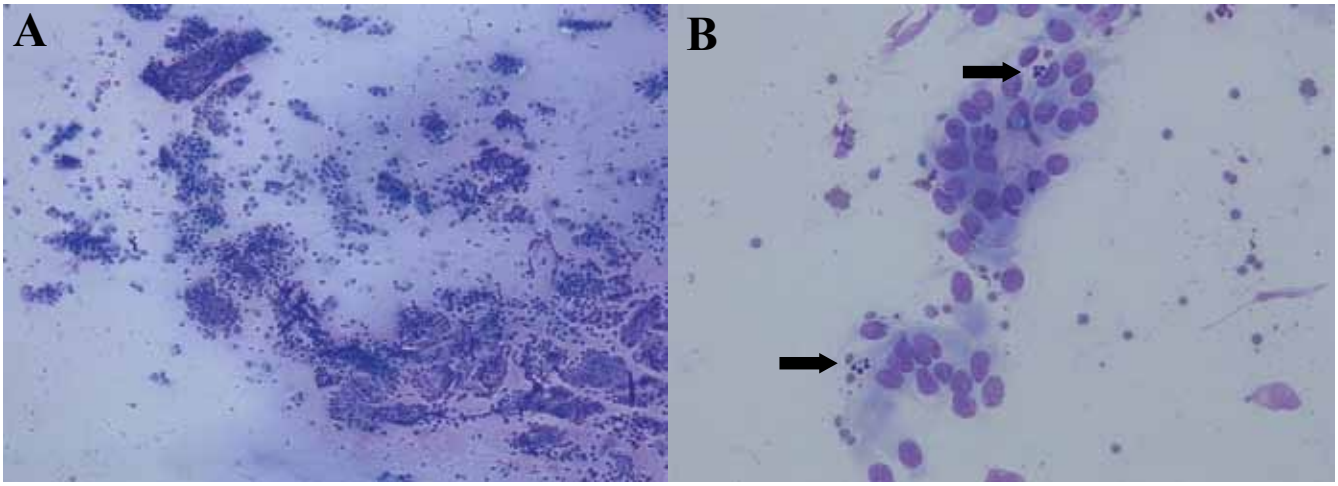

Figure 3. Endometrial cytology smears stained with Diff-Quick®, observed by light microscope. A. 100x, B. 400x (arrows point at polymorphonuclear cells).

al., 2006). On the other hand, the cytologic sampling technique "low volume uterine flushing" was firstly described by Ball et al. (1988) in mares, and subsequently modified by Gilbert et al. (1992; 2005) for its use in cows. However, CB and LVL lavage techniques are essentially based on different approaches (Pascottini et al., 2016b). Each technique has particular advantages and disadvantages. For example, when the uterine cavity is flushed with the liquid infused during LVL sampling, it harvests cells and debris present in the uterine lumen, and probably the superficial layer of the endometrial epithelium (Kasimanickam et al., 2005b) sampling the majority of the uterine surface. The CB technique on the other hand only samples a very limited portion of the endometrium, and loose PMNs are therefore only locally collected (Pascottini et al., 2016b).

Cytotape: a novel cytological sampling technique to diagnose cytological endometritis in dairy cows

Although the importance of CYTO on the fertility and profitability of dairy farms is largely known, standardization of endometrial cytology diagnosis in dairy cows has not been fully established yet (Pascottini et al., 2015) (Table 2). In different publications, the time relative to calving the samples were taken varied from 21 to 64 DIM (Kasimanickam et al., 2004; Gilbert et al., 2005; Hammon et al., 2006; Barlund et al., 2008). Concomitantly, PMN threshold levels to diagnose CYTO ranged from 3 to 18% (Salasel et al., 2010; de Boer et al., 2014) resulting in a wide variation (9 to 76%) in reported CYTO prevalence (Barański et al., 2012). Consequently, comparing results between studies is almost unfeasible. However, a novel technique, cytotape (CT), was developed to take endometrial samples at the moment of AI (Pascottini et al., 2015). Cytotape consists of a 1.5 piece of paper tape rolled on the tip of a standardly loaded insemination gun, covered with a double guard sheath. This simple technique has achieved high cytology standards when compared with the cytobrush, its main advantage being the possibility to sample cows during AI by using ordinary material (Pascottini et al., 2015) (Figure 4). Sampling during AI obviously has three significant benefits: (1) standardization of the moment of sampling and assessment of the uterine health status at the most critical point, being the moment of insemination; (2) allowing the use of a universal PMN cutoff point, as the moment of sampling is standardized; and (3) no extra manipulation of the animal is required, as CYTO sampling and AI are performed simultaneously. However, it may be arguable that sampling during estrus might lead to false-positive results since a higher PMN infiltration during estrogenic dominance (histopathology) has been reported (Ohtani et al., 1993; Subandrio et al., 2000). Still, Madoz et al. (2013) suggested that the physiological infiltration of PMNs was not related to the stage of the estrous cycle, at least in studies based on cytologic samples. This is in concordance with the results obtained by the authors (Pascottini et al., 2016a, Pascottini et al., 2016c), where the absence of PMNs in a high number of cytology samples harvested during AI was demonstrated.

In two large field studies performed by the research group of the Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine (Ghent University) using the cytotape device, a universal PMN cut off point of 1 % in cytology samples achieved during AI was established in both dairy cows and nulliparous dairy heifers (Pascottini et al., 2016a; Pascottini et al., 2016c). More than one quarter of the modern dairy cows that are inseminated, suffer from CYTO, with a substantial detrimental effect on fertility (conception rate in CYTO negative samples 47% versus 32.7% in CYTO positive samples). As expected, the prevalence of CYTO was low (7.9%) in nulliparous dairy heifers. This may probably be attributed to the fact that these animals did not experience a prior parturition and concomitant damage and bacterial contamination. However, in nulliparous dairy heifers, the conception rate in CYTO negative samples was 62.8% while it was only 38.5% in CYTO positive samples. Risk factors associated with CYTO prevalence were different in cows versus nulliparous heifers. In cows, risk factors associated with CYTO occurrence were parity ≥ 2, DIM at AI ≥ 124, and warm months of the year. On the other hand, the only risk factor associated with CYTO occurrence in nulliparous heifers was the performance of a previous unsuccessful insemination. These studies clearly demonstrate the deleterious effect of CYTO diagnosed at AI and the importance of its diagnosis on a regular basis in order to set up specific management decisions and eventually specific treatments.

Innovative perspectives in CYTO diagnosis and treatment

The examination by CT during first insemination allows the exploration of uterine health without any extra labor cost. If the breeding is not successful and the cow is diagnosed CYTO positive, it will be possible to set up a more targeted treatment to increase the odds of pregnancy at a subsequent AI. The use of this new tool could furthermore be interesting when inseminating and simultaneously sampling repeat breeder cows, aiming to more accurately diagnose the underlying reason of the repeat breeding. However, it is important to mention that currently no consensus exists concerning an effective CYTO treatment. The use of PGF2α during diestrus results in luteolysis and, subsequently, estrus, which has been suggested to enhance local immunity by removal of the immunosuppressive effect of progesterone (Lewis, 2003, Lewis, 2004). Furthermore, PGF2α is claimed to possess an ecbolic effect, by which it effectuates the elimination of bacteria and debris (Lima et al., 2013). However,

Table 2. Summary of subclinical endometritis studies around the world, considering different time points postpartum, thresholds for percentage of polymorphonuclear cells and respective prevalence.

Reference	Country	Days in milk	PMN (%)	Prevalence (%)
Kasimanickam et al. (2004)	USA	20-33	>18	45
		34-47	>10	41
Dubuc et al. (2010)	Canada	35±3	≥6	13.5
		56±3	≥4	9.6
Plöntzke et al. (2010)	Argentina	18-38	>5	38
		32-52	>5	19
McDougall et al. (2011)	New Zealand	29±2.4	≥9	29
		43±2.3	≥7	23
Bogado Pascottini et al. (2016)	Belgium	>60	≥1	27.8

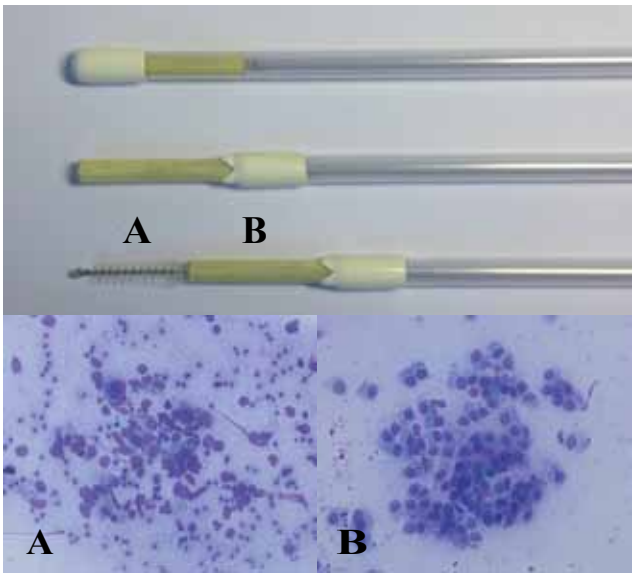

Figure 4. A. Cytobrush sample with fragmented cells and moderate red blood cell contamination. B. Cytotape sample exhibiting good quality (intact) endometrial cells.

only one study showed a significant benefit of PGF2α treatment on CYTO prevalence (Kasimanickam et al., 2005a), whereas other studies did not (Galvão et al., 2009a, Lima et al., 2013). Some authors unsuccessfully tried to correlate the presence of CYTO with bacterial infection (McDougall et al., 2011; Barański et al., 2012; Madoz et al., 2014). However, others have demonstrated the benefits of an intrauterine antibiotic treatment (Kasimanickam et al., 2005a, Denis-Robichaud and Dubuc, 2015b), but not Galvão et al. (2009b). Recently, The authors of this review demonstrated the possibility to reduce the amount of PMNs present in the uterine lumen by applying a uterine lavage with 500 to 600 mL of a sterile saline solution (Dini et al., 2015). However, further field studies are necessary to confirm this as a potential treatment for repeat breeder cows suffering from CYTO. Contradictory results have been put forward regarding the application of non-steroidal anti-inflammatory drugs following AI (Hirsch and Philipp, 2009; Erdem and Guzeloglu, 2010; Heuwieser et al., 2011). However, such a treatment specifically targeted for cows suffering from CYTO diagnosed at AI could represent a valid strategy to improve the pregnancy outcome of that AI.

CONCLUSION

Approximately 50% of dairy cattle develop uterine disease after parturition. In this review, the latest clinical definitions and the most efficient therapeutic approaches of the postpartum uterine disease complex were exposed. However, the main aim of farmers and practitioners should be focused on the prevention of the risk factors associated with uterine diseases. More research is needed in order to reduce the uterine diseases prevalence by applying strategic management decisions on nutrition, genetics and preventive medicine.

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A comprehensive reference list is available upon request.

Inhoud van de 85ste jaargang 2016

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THEMA: ZIEKTEVEROORZAKENDE MUTATIES BIJ HONDEN

THEMA: HYPERTHYREOÏDIE BIJ KATTEN

OVERZICHTSARTIKELS

CASUSREEKS

VOOR DE PRAKTIJK

SHORT COMMUNICATION

PERMANENTE VORMING

UIT HET VERLEDEN

VRAAG EN ANTWOORDRUBRIEK

Contents volume 85, 2016

Vlaams Diergeneeskundig Tijdschrift

ANESTHETICA VOOR OVARIËCTOMIE BIJ DE KAT

VRAAG

"Voor de sterilisatie van een volwassen kattin gebruiken we standaard medetomidine en ketamine als verdoving. We voorzien ook een catheter om een IV-weg te garanderen. Indien de patiënt tegen het einde van de operatie oppervlakkiger in anesthesie is, hebben we de gewoonte om een kleine dosis alfaxalone IV toe te dienen (bijvoorbeeld om wakker worden tijdens intradermale hechting te voorkomen). Is dit een goede/veilige gewoonte of is het beter om terug hetzelfde anestheticum toe te dienen en geen "mix" te maken?"

ANTWOORD

Ovariëctomie bij de kat is een korte chirurgische ingreep, waarbij de anesthesie courant gebeurt met een intramusculaire injectie van (dex)medetomidine en ketamine. Deze combinatie biedt zowel anesthesie, spierrelaxatie en analgesie, wat resulteert in een gebalanceerd anesthetisch protocol met een te verwachten anesthesieduur van ongeveer 30-40 minuten. Deze tijdsspanne is normaal gezien lang genoeg om electieve chirurgische ingrepen te doen. Het is echter steeds verstandig om een intraveneuze toegangsweg te voorzien, mocht er zich een probleem voordoen tijdens de anesthesie. Bij een plotse nociceptieve reactie op een chirurgische prikkel kunnen op die manier bijkomende analgetica, zoals bijvoorbeeld buprenorfine of methadon intraoperatief toegediend worden. Op dezelfde manier kan een bijkomende dosis van een kortwerkend anestheticum worden toegediend. Bijvoorbeeld wanneer de patiënt plots te oppervlakkig in anesthesiediepte is, waarbij de voorkeur dan uitgaat naar een getitreerde dosis (lees: op effect) van een kortwerkend, niet-cumulerend intraveneus anestheticum. Hierbij wordt de recovery van de patiënt minimaal beïnvloed. Alfaxalone is daarom een goede keuze (de werkingsduur bedraagt een tiental minuten). Bovendien heeft het ook cardiovasculair stabiele eigenschappen. Het nadoseren met ketamine is ook een optie, maar door zijn langere werking en zijn cumulerend effect bij herhaalde dosering verlengt dit zeer waarschijnlijk de recovery van de kat.

Het toedienen van een tweede anestheticum wanneer het eerste uitgewerkt is, is dus een optie mits de dosis wordt aangepast aan de reële behoefte van de patiënt op dat moment.

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AFWEZIGHEID VAN KEURDERS VÓÓR HET SLACHTEN

VRAAG

"Hoe verloopt de procedure als een bedrijf volgens het opgegeven slachtplan niet kan opstarten omdat er geen keurders (ante- en postmortem) beschikbaar zijn? Ik denk hierbij aan ongevallen op weg naar het bedrijf of verkeerssituaties. Ik weet dat het bedrijf zijn activiteiten niet mag aanvangen maar wat met de verloren arbeidsuren van het personeel dat wel beschikbaar is?"

ANTWOORD

Overeenkomstig de verordening 854/2004 van het Europese parlement en de Raad van 29 april 2004 houdende vaststelling van specifieke voorschriften voor de organisatie van de officiële controles van voor menselijke consumptie bestemde producten van dierlijke oorsprong artikel 5, punt 1 en bijlage I, sectie I, hoofdstuk II, punt B moet de officiële dierenarts (= de keurder) vóór het slachten alle dieren aan een antemortemkeuring onderwerpen. Dit betekent dat de slachtactiviteiten pas kunnen starten, nadat de dieren onderworpen werden aan een dergelijke keuring. Indien geen antemortemkeuring werd uitgevoerd, dienen de betrokken dieren ongeschikt verklaard te worden voor menselijke consumptie overeenkomstig bijlage I, sectie II, hoofdstuk V, punt 1a van dezelfde verordening.

Omtrent de gevolgen voor het slachthuis (zoals verloren arbeidsuren) bij de te late aanwezigheid van de officiële dierenarts is er bij mijn weten geen procedure door het FAVV voorzien.

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RIBFRACTUREN BIJ KOEIEN

VRAAG

"In het slachthuis worden bij melkkoeien van elke leeftijd zeer frequent geheelde fracturen opgemerkt van voornamelijk de derde en de vierde rib. Dit soms bilateraal. Wat kan hiervan de oorzaak zijn?"

ANTWOORD

Ribfracturen bij melkkoeien krijgen recentelijk meer aandacht in het kader van dierenwelzijn. Bij oudere koeien en vooral bij oudere, chronisch manke koeien worden voornamelijk fracturen ter hoogte van costochondrale overgang van de zevende tot negende rib gerapporteerd. Deze fracturen zijn bij het levende dier op te merken als een harde en soms pijnlijke zwelling ongeveer een handbreedte achter het olecranon (Blowey en Weaver, 2011). Fracturen van het kraakbenige gedeelte van de valse ribben worden ook vermeld. Op deze letsels kan callusvorming ontstaan met een blijvend pijnlijke zwelling. Als oorzaak van deze fracturen wordt aangegeven dat manke koeien minder soepel gaan neerliggen, waarbij de hardere impact met de bodem een plotse druk op de ribbenboog veroorzaakt met fracturen ter hoogte van het zwakste punt, zijnde de costochondrale overgang en de minder goed gefixeerde valse ribben. Onvoldoende zachte bedding zou hierbij het risico op fracturen verhogen. In een Engelse studie van tweeduizend koeien op dertien bedrijven werd een gemiddelde prevalentie van 15,8 % (3,6 tot 26,8%) gevonden (Boyling, 2011; Blowey en Bell, 2014). Daarbij werd ook een significant hogere prevalentie bij manke koeien gevonden.

Ribfracturen van de tweede tot vijfde rib worden ook als slachthuisbevinding beschreven. Deze meestal geheelde fracturen zijn hier vooral ter hoogte van het riblichaam te vinden, ofwel in het midden van de rib, ofwel 10 tot 20 centimeter boven de costochondrale overgang (Blowey en Bell, 2014; Patton, 2014).

De hypotheses over de oorzaak variëren: Patton (2014) suggereert een trauma als kalf tijdens groepshuisvesting, terwijl Blowey en Bell (2014) verwijzen naar een studie van Maddox uit 1986 waarin een verband tussen ribfracturen ter hoogte van de tweede tot vierde rib en een lage conditiescore van oudere koeien aangetoond werd. Over een mogelijk verband met demineralisatie of andere metabole stoornissen, zoals bijvoorbeeld rachitis als oorzaak van ribfracturen bij kinderen, is in de literatuur niets te vinden

Samengevat kan gesteld worden dat oude ribfracturen bij melkkoeien inderdaad een frequente slachthuisbevinding zijn en dat meer onderzoek nodig is om de exacte oorzaak te bepalen. Vooral het mogelijke verband met conditiescore en mankheid kan erop wijzen dat de ribfracturen een verklikker van verminderd dierenwelzijn zijn.

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STAANDE CASTRATIE VAN HENGSTEN

In de tijd dat anesthesie in praktijkomstandigheden nog quasi onmogelijk was, waren ‘castreurs’ actief die gespecialiseerd waren in het ‘staand’ castreren van hengsten. Weliswaar kon men paarden ‘omleggen’ en met boeien vakkundig vastkluisteren. Eén been werd naarvoor getrokken om de balzak optimaal te exposeren. Dat omleggen was echter niet zonder risico en werd door de eigenaars ongaarne gezien. Kantelende operatietafels waren dure constructies en bestonden enkel aan de veeartsenijscholen.

Een bekende castreur was wijlen Ernest (Nesten) Ollivier uit Kuurne, werkzaam in België en Noord-Frankrijk, vooral in het draversmilieu. Wat hier volgt is een ooggetuigenverslag van de manier waarop de man te werk ging.

Hij spoot de hengst vooraf in met combelene (1ml per 100 kg L.G.) intramusculair in de pectoraalspieren. Dat gebeurde dus niet in de nekspieren om te vermijden dat het paard bij mogelijke infectie met abcesvorming een stijve nek zou overhouden.

Intussen nam de castreur een gewone huishoudemmer, vulde hem halfvol met lauw water en deed daar een scheut creoline in. Op de rand van deze emmer stak hij dan twee zelfgemaakte houten klemmen vast, die aan één zijde samengeknoopt waren en waarvan de binnenkant ingestreken was met een adstringerende (10% kopersulfaat) zalf. In diezelfde emmer legde hij ook zijn bistouri (dit was eigenlijk een aangepast zakmes dat gemakkelijk toe klapte om bij elke onverwachte beweging van het paard niets te kwetsen) en ook een lichte,

De foto toont het eigen materiaal van Ernest Ollivier, overgemaakt aan Dr. Gunst en door deze laatste geschonken aan de Museumcollectie Veterinair Verleden Merelbeke, waarvoor onze bijzondere dank (L.D.)

handige, zelfgemaakte tang waarmee hij de klem, die over de zaadstreng zou worden geschoven, kon toenijpen.

Vervolgens plaatste hij een ijzeren neusnijper op de neus van de reeds gesedeerde (lichtjes uitlangende) hengst. Dit was een speciale praam die, eenmaal op de neus gezet, er niet meer kon afglippen! Een helper moest het touwtje aan de nijper vasthouden en er indien nodig een beetje mee schudden. Ook werd die helper gewaarschuwd op te letten voor de voorpoten van het paard, voor het zogenaamde ‘stekken’.

Toen was het moment van de castratie gekomen. Nesten plaatste zich dan tegen de linkerflank van de hengst, 'schoorde' zich letterlijk met hoofd en linkerschouder, de benen een beetje gespreid, in de linkerflank van het paard. Hij fixeerde met zijn linkerhand de linkerteelbal naar beneden en met het zakmes in de andere hand kliefde hij in één k(l)ap over 5 à 10 centimeter lengte doorheen huid, subcutaan bindweefsel én Tunica dartos. Zo hing de testikel bloot. Hij stak een middenvinger in het zakje gevormd door de omgeslagen tunica en duwde met de andere hand het bindweefsel rond de zaadstreng omhoog en los. Van achter naar voor schoof hij toen een van de twee houten klemmen over de vrijgemaakte zaadstreng. De tang griste hij uit de emmer om op het voorste uiteinde van de klem te plaatsen en kneep die toe (het andere, achterste uiteinde van de klem werd dichtgehouden door een touwtje). Dat toenijpen deed de castreur altijd in twee tijden, niet alleen omdat dit zeer pijnlijk is, maar ook omdat het toch wat tijd vroeg om de meer dan één centimeter dikke zaadstreng tot een papierdunne streng te reduceren. Tot slot plaatste hij met een touwtje een mastknoop op het voorste deel van de klem, verwijderde de tang en sneed (enkel) de testis af.

De castratie van de rechtertestikel gebeurde op dezelfde wijze aan de rechterzijde van het paard. Antitetanosserum werd toegediend, de neuspraam afgenomen en de rest van de creolineoplossing werd tussen de achterbenen van de paard gegoten ter ontsmetting.

Dat alles gebeurde met een bewonderenswaardige snelheid en accuraatheid. Naar eigen zeggen kon Nesten op een voormiddag enkele tientallen bijeengebrachte hengsten castreren. Dat betekende echter niet dat alles en altijd zomaar probleemloos verliep. Op de vraag of hij soms klop kreeg, antwoordde Nesten simpelweg: ‘Ja, veel, en overal’. Zelfs met een dreigende liesbreuk kon hij overweg. Minstens één keer wist hij nog snel het zaakje terug te duwen en de huid vast te zetten met een castratieklem op beide lippen van de snijwonde. De klem werd extra bevestigd met twee hoefnagels doorheen de huidflappen er onder gestoken. Daarna werd het paard naar de ‘school’ gevoerd om een en ander in betere omstandigheden en met beter materiaal te hechten.

Omer Gunst

met dank aan Paul Desmet

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Profiel:

Wij zoeken een dierenarts met goede kennis en vaardigheden, maar vinden de omgang met de klant en dier in eerste instantie het belangrijkste. We verwachten dat onze nieuwe dierenarts uitblinkt in communicatieve vaardigheden en een teamspeler is. Diergeneeskundig verwachten we dat de kandidaat streeft naar de beste diergeneeskundige zorg voor het dier en dat hij of zij zich verdiept in een van de disciplines. Ook dierenartsen die zich al verder ontwikkeld hebben, die een leuke en inspirerende werkplek zoeken, worden uitgenodigd te solliciteren.
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12 WEKEN BESCHERMING

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