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INHOUD

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Wat een fantastisch zicht toch: de vrijheid van een **kat** in al zijn glorie. Hoe ze soms uren vreedzaam in de zon kunnen liggen slapen, maar evengoed enkele uren later zonder moeite met de levens van muisjes spelen. Helaas gaat deze vrijheid geregeld gepaard met een verhoogd risico op allerlei aandoeningen, zoals parasitaire infecties, aanrijdingen, kattenaids en vechtabcessen. Deze vechtabcessen kunnen in sommige gevallen leiden tot een etterige ontsteking van de borstholte of pyothorax, die potentieel dodelijk kan zijn. In dit VDT-nummer worden risicofactoren en plan van aanpak van pyothorax uitgebreid besproken aan de hand van twee klinische gevallen en een overzicht van de meest recente aanbevelingen in de literatuur.

Tekst: Falke Gorris

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Negative pressure wound therapy: the past and the future

Negatieve druktherapie: het verleden en de toekomst

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ABSTRACT

Negative pressure wound therapy (NPWT) involves the application of negative pressure on a wound bed for its positive effects on wound healing. Indications for NPWT concern various types of wounds, skin grafts and flaps, partial-thickness burns, open abdomen management and closed incisions.

Negative pressure wound therapy has been used for centuries in human medicine. Its first use dates back to the Roman era (around 27 BCE) when human generated pressures were used. Later, European and Russian physicians developed various advanced methods and systems to apply negative pressure on wounds or other injuries. The on-going positive findings in human medicine triggered researchers in veterinary medicine to apply this technique on animal patients.

However, much still has to be investigated regarding NPWT, especially in veterinary medicine, as there are many factors playing a role in the mechanisms of this treatment. New methods and techniques are continuously being developed and the existing studies show great potential for NPWT.

SAMENVATTING

Bij negatieve druktherapie wordt het wondbed onderworpen aan een negatieve druk omdat dit positieve effecten heeft op de wondheling. Negatieve druktherapie kan worden toegepast op verschillende soorten wonden, huidtransplantaten en -flappen, open abdomen en gesloten incisies.

Negatieve druktherapie wordt al eeuwen gebruikt in de humane geneeskunde. Al in de Romeinse tijd (rond 27 v.Chr.) gebruikte men door mensen gegenereerde zuigkracht om verwondingen beter te laten helen. Europese en Russische artsen ontwikkelden later verschillende geavanceerde methoden en systemen om negatieve druk op wonden toe te passen. De positieve bevindingen in de humane geneeskunde wakkerden de interesse van onderzoekers in de diergeneeskunde aan. De laatste twintig jaar wordt negatieve druktherapie bij dieren ingezet in talrijke experimentele en klinische omstandigheden, maar veel onderzoek moet echter nog gedaan worden.

Anderzijds worden voortdurend nieuwe technieken ontwikkeld en de bestaande studies voorspellen veelbelovende toekomstmogelijkheden voor negatieve druktherapie in de diergeneeskunde.

INTRODUCTION

Wound healing can be divided into different phases (Buchanan et al., 2014; Balsa and Culp, 2015). The first phase is the inflammatory phase, which comprises a vascular response for hemostasis and a cellular response with leukocyte infiltration. Subsequently, the proliferation phase starts, defined by fibroplasia, angiogenesis and epithelization. The initial part of this phase is hallmarked by the formation of fibroblasts, collagen and new blood vessels, presenting clinically as granulation tissue. After the cellular proliferation, wound contraction is initiated and in combination with reepithelialization, the wound bed becomes smaller. In the last phase, the formation of scar tissue occurs, which is called remodelling. This final phase of wound healing may last for months or even years.

From a surgical point of view, there are different approaches to wound closure. With primary or delayed primary closure, the joining of wound edges before granulation tissue formation is attempted and it is applicable to acute, non-contaminated or minimally contaminated wounds. It results in 'per primam' healing. When wounds cannot be closed with primary closure because of the large size or degree of contamination, they can be allowed to close by second intention healing. Wound healing will pass all phases and is called 'per secundam' healing. Surgically closing a wound after the formation of granulation tissue is called 'per tertiam' healing, and indications involve chronic, contaminated and infected wounds (Knapp-Hoch and de Matos, 2014; Balsa and Culp, 2015). In wounds that do not allow primary closure, various wound treatments can be exploited to optimize wound healing and shorten the time before definitive wound closure may be considered. One of these approaches

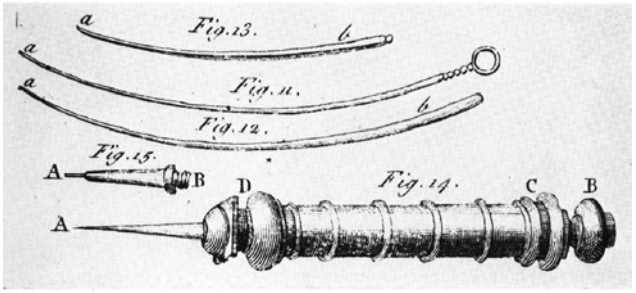

Figure 1. Drawing of an Anel syringe (Price, 1969).

is negative pressure wound therapy (NPWT). Negative pressure has been used for centuries in the treatment of various wounds and diseases in human patients (Miller, 2012). Already during the Roman era (around 27 BCE), negative pressure was applied on wounds in the form of human-generated pressures (Price, 1969; Miller, 2012). With time, instruments and devices were invented to generate more suction and to control the pressure applied to the patient in a better way (Howe, 2015).

Over the last twenty years, NPWT has been further developed in both human and veterinary medicine. Although animals were mostly used as study models for human medicine, the advantages indicated by the literature in human medicine have recently attracted the attention of researchers in the veterinary field (Howe, 2015).

In recent years, NPWT has already been the topic of other articles published in Vlaams Diergeneeskundig Tijdschrift; besides a review article on the mechanics of NPWT (Spillebeen et al., 2013), three case reports are available (Jordana-Garcia et al., 2011; Abma et al., 2015; Lippens et al., 2016). The current article provides an overview of the history of NPWT and zooms in on the future perspectives of this treatment modality, which could further contribute to the development and refinement of the technique in veterinary medicine. In this review, the term NPWT will be used when referring to the treatment with negative pressure.

HISTORY OF NEGATIVE PRESSURE WOUND THERAPY

Human medicine

Negative pressure wound therapy originated in human medicine during the Roman era (around 27 BCE), when people who were thought to have healing powers, used their mouth to apply negative pressure to the wound bed. This type of therapy was called 'human lip service' (Price, 1969; Miller, 2012). During the same era, also heated cupping glasses were used to exert a subatmospheric pressure on wounds to drain fluids. As the cups cooled down, the pressure decreased and negative pressure was attained (Miller, 2012).

In the 18th century, the direct mouth suction was abandoned because it was considered to be repulsive and unpleasant (Price, 1969). The French surgeon, Dominique Anel, was inspired by this ancient technique of wound suction and invented a syringe that mimicked the action of wound suction (Figure 1). It could drain fluids from abscesses, hematomas, deep puncture wounds and even infected sinuses (Price, 1969).

Later, cupping became predominant in NPWT. The 'Glass Leech' was developed in 1821 by Francis Fox, a British physician. This device looked like a leech and presumably drew blood into the suction site (Miller, 2012; Museum of Health Care, 2015) (Figure 2). Thereafter, cups with different shapes and sizes were developed, including associated tubes and bulbs. In this way, exudates from various locations of the body could be extracted (Miller, 2012).

In 1977, Patrick Sames described the use of vacuum by surgeons to drain wounds and the importance to cover these areas. He found a less expensive way to seal these areas by using cling film. It was a new, effective and cheaper way to seal the wound bed for NPWT (Sames, 1977). Around the same time, more articles were published about the effectiveness of vacuum therapy. For example, in 1986, the Russian surgeon, Nail Bagaoutdinov, described a so-called 'vacuum aspiration system' with foam dressing and an irrigation tube for infected wounds. This allowed cleansing and suction of the wound at the same time (Couch and Stojadinovic, 2011; Daar et al., 2016). He tried to get a patent on his technique and device but it was rejected because it looked too much like the Bier Cup, which was a glass cup linked to a manual pump and was used around the 1890's (Otterbourg, 2012). The advantages of the 'vacuum aspiration system' were the removal of secretions with cleansing of the wound and a rapid detoxification (Lockwood et al., 2004). For the first time, with NPWT, it was possible to place mechanical tension on soft tissues and thus provide a growth stimulus for fibroblasts (Nolff et al., 2015c).

As more beneficial effects were discovered, new techniques were proposed. Svedman et al. (1986) described the simultaneous use of suction pressure and continuous or intermittent irrigation of the wound (Svedman et al., 1986). Chariker et al. (1989) proposed a NPWT system with the use of moist medical gauze instead of the customary foam, at a pressure of -80 mmHg. The authors stated that moist gauze avoided the presence of a dead space and minimized eschar formation (Chariker et al., 1989).

In 1993, Fleischmann et al. reported the use of NPWT to treat wounds of fifteen patients with open fractures that were covered by polyvinyl foam, a polyurethane dressing, and a suction device. It resulted in well-cleaned wounds and distinct granulation tissue formation (Fleischmann et al., 1993). Up until this study, the results of NPWT were evaluated by visual aspects, which by nature is subjective. The following years, various methods to measure changes in the wound bed were developed, such as measuring blood

flow by Laser Doppler (Morykwas et al., 1997), or radioisotope perfusion imaging (Kairinos et al., 2009). These objective methods helped to reveal the effects of NPWT on wound healing.

The commercialization of the modern NPWT system was first done by Kinetic Concepts, Inc., USA (KCI) in 1995, who derived the technique from a large-scale clinical trial of Argenta and Morykwas and called it vacuum-assisted closure (V.A.C.) therapy system (Argenta and Morykwas, 1997). Thereafter, in 1996, Fleischmann developed an intermittent instillation NPWT technique (NPWTi) combining NPWT with wound lavage therapy (Fleischmann et al., 1998). The NPWTi system evolved the following years into an automatic system and in 2001, KCI produced a fully electric device with an automatic electric pump and alarm functions (V.A.C. Instill) to warn when abnormalities occurred (Lehner, 2009). The V.A.C. ultra therapy is the most recently developed system (Kim et al., 2013). With this system, specific settings can be managed, i.e. instil volume, soak time and the time, in which negative pressure is applied between instillation phases (KCI Licensing Inc.).

Benefiting from the pioneering work of KCI, other companies started to develop their own devices, and commercialized these at a lower cost compared to the V.A.C. device (Otterbourg, 2012; Garwood and Steinberg, 2016). Currently, fourteen different manufacturers have commercialized NPWT devices, each providing different types of devices (Glass and Nanchahal, 2012; Dumville et al., 2015).

Veterinary medicine

During the 20th century and well into in the 21st century, animal studies related to NPWT were mostly performed to support scientific findings in human medicine (Morykwas et al., 1997; Norbury and Kieswetter, 2007; Jacobs et al., 2009; Suh et al., 2016; Tanaka et al., 2016). Morykwas et al. (1997) were the first to set up such animal studies, and used twenty-five pigs to examine the acceleration of wound healing by secondary intention with the V.A.C. device and methodology. Specifically, they examined the effects on blood flow and delivery of nutrients, the development of granulation tissue at continuous and intermittent subatmospheric pressure, and bacterial colonization (Morykwas et al., 1997).

The first reported veterinary case treated with NPWT concerned a tiger of six weeks old with multiple wounds (Lafortune et al., 2007). When conventional wound therapy had failed after dehiscence of a skin flap, NPWT was applied. The authors proposed that the technique could be beneficial in the treatment of complex and chronic wounds in domestic animals, neonatal zoo animals and reptiles (Lafortune et al., 2007).

In the past years, more studies and case reports of various species, such as cats (Guille et al., 2007; Owen et al., 2009; Nolff and Meyer-Lindenberg, 2015a; Nolff and Meyer-Lindenberg, 2015b), dogs (Ben-Amotz et al., 2007; Israel and Hickman, 2011; Buote and Havig, 2012; Cioffi et al., 2012; Spillebeen et al., 2013; Stanley et al., 2013; Pitt and Stanley, 2014; Abma et al., 2015; Coutin et al., 2015; Nolff et al., 2015a; Nolff et al., 2015b; Nolff et al., 2015c; Or et al., 2015; Lippens et al., 2016; Miller et al., 2016; Or et al., 2016), horses (Gemeinhardt and Molnar, 2005), and even birds (Knapp-Hoch and de Matos, 2014), tigers (Lafortune et al., 2007), tortoises (Adkesson et al., 2007), and a rhinoceros (Harrison et al., 2011) were published. Negative pressure wound therapy has been used to accelerate wound closure, stimulate granulation tissue production, immobilize skin grafts, and remove and prevent fluid accumulation (Ben-Amotz et al., 2007; Guille et al., 2007; Stanley et al., 2013). It effectively reduced and closed dead space (Knapp-Hoch and de Matos, 2014), promoted wound contraction, and resolved infection (Ben-Amotz et al., 2007; Nolff et al., 2015c). It has been used in conjunction with flap surgery (Nolff and Meyer-Lindenberg, 2015b; Or et al., 2015), orthopedic complications (Bertran et al., 2013), necrotizing fasciitis (Abma et al., 2015) and septic peritonitis (Buote and Havig, 2012; Cioffi et al., 2012). Overall, animals have a high tolerance for NPWT and there have been numerous applications in veterinary medicine (Nolff and Meyer-Lindenberg, 2016).

FUTURE PERSPECTIVES IN VETERINARY MEDICINE

Negative pressure wound therapy appears a promising technique for the treatment of complicated wounds. Negative pressure wound therapy has a history of research and development (Figure 3), but further research is necessary to optimize the technique and its applications in practice, both in human and veterinary medicine. One of the difficulties of comparing the existing literature on NPWT is that outcome measures of NPWT are different, which contribute to reported con-

Figure 2. Glass cupping set of Dr. Fox from around 1850 (Anonymous, 2015).

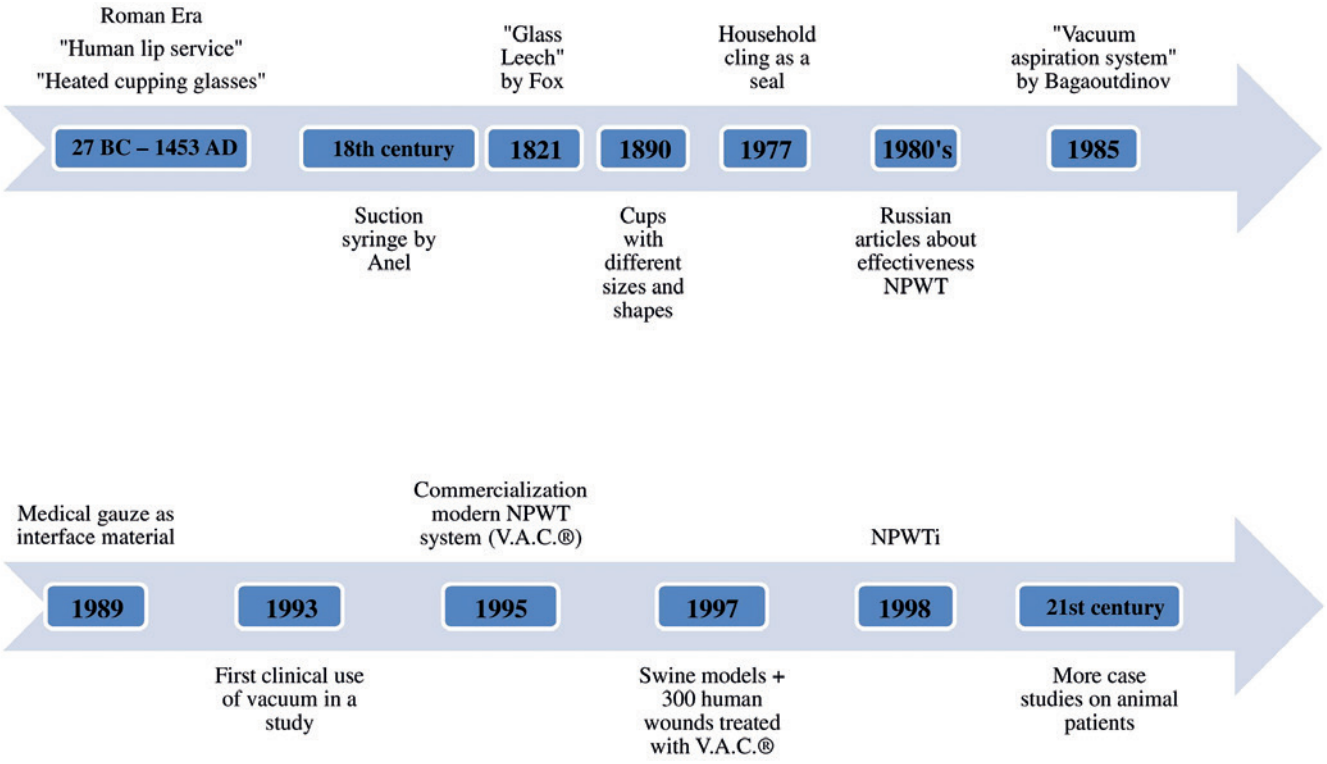

Figure 3. Historical timeline of the use of NPWT.

troversial results. Even when measurement methods could be standardized, it will still remain difficult to objectively compare results, as traumatic wounds are unique. Furthermore, there is a lack of blinded, double-blinded and meta-analysis studies concerning NPWT. Blinding for the treatment protocol is often not possible because wounds and periwound environment show clear evidence of NPWT, predisposing to a bias during wound evaluation (Moues et al., 2007). Furthermore, meta-analysis in the veterinary literature is often not possible due to the relatively limited amount of cases.

Although less important for research purposes but clinically relevant, is the fact that NPWT systems have undergone quite some development. For veterinary use, the V.A.C. simplicity system and the V.A.C. freedom portable devices have recently been introduced (KCI® Animal Health, 2016). Moreover, in an Australian study, the first use of the portable PICO™ pump has been described (Miller et al., 2016). This is of great advantage in the veterinary field since incorporation of the vacuum pump into a harness allows patient movement without termination of treatment (Pitt and Stanley, 2014; Coutin et al., 2015; Miller et al., 2016; Nolff and Meyer-Lindenberg, 2016). In this way, NPWT could be used on a home treatment basis, provided that owners are competent enough to manage and closely monitor the system and the health status of their pet. Owners can be trained for home management, reducing the need for a doctor visit to change dressings or when complications are suspected. Prolonged hospitalization and its associated costs can thus be avoided, making pet owners potentially more in favor of performing NPWT (Pitt and Stanley, 2014). It should be emphasized that the consumables used for NPWT are rather costly.

The advent of less bulky NPWT devices might also stimulate the use of NPWT in cats. So far, the use of NPWT in cats has been described in several case reports, a retrospective cohort study and a clinical case series (Guille et al., 2007; Owen et al., 2009; Nolff and Meyer-Lindenberg, 2015a; Nolff and Meyer-Lindenberg, 2015b; Nolff et al., 2016). Currently, there are no experimental studies. More studies in cats would be interesting, for it is believed that NPWT could have the same positive effects in cats as in dogs (Nolff and Meyer-Lindenberg, 2016).

Pressure setting is one of the most studied parameters in various applications of NPWT. Most devices allow the pressure to be set at different values. However the majority of the commercial devices measure the pressure at the pump, while the actual pressure at the wound would be the preferred location for pressure measurement, since this can differ significantly from the pressure at the pump (Nolff and Meyer-Lindenberg, 2016). Pressure recommendations proposed in human medicine have a therapeutic range of -40 to -150 mmHg (Birke-Sorensen et al., 2011). A pressure of -125 mmHg has been suggested as the ideal pressure for optimal blood flow and granulation tissue production in animal wounds (Morykwas et al., 2001), although other studies found similar or better effects with other pressure values (Borgquist et al., 2010; Birke-Sorensen et al., 2011).

In addition to the location of pressure sensing, the transmission of pressure along the wound bed also depends on the interface material used (Or et al., 2016). Along with the development of the NPWT systems, different contact layer materials have been developed, i. e. foams, gauzes, impregnated materials and adhesive or non-adhesive sheets, each having their own recommended indications. Oxidized regenerated cellulose is thought to be a new interface material for NPWT (Glass and Nanchahal, 2012). It adequately drains exudates, it provides inactivation and removal of matrix metalloproteinases, and it absorbs and modulates growth factor release (Glass and Nanchahal, 2012).

Furthermore, it is currently investigated in an interventional study whether a novel contact layer called Bio-Dome (ConvaTec, USA), linked to the Engenex system, is useful in human NPWT patients. Bio-Dome creates space above the wound surface, which prevents ingrowth of granulation tissue into the interface material, and keeps its integrity after initiating the therapy. It seems to have comparable positive effects on wound healing as NPWT systems at the standard pressure of -125 mmHg, and even with less hypobaric pressure (Hoeksema, 2015). Currently, research is still ongoing.

Further treatment improvements may be expected by combining other wound treatment approaches with NPWT. Scaffolds, such as Integra (Integra Lifesciences Corporation, USA), an artificial dermis, have been examined in animal models and in human patients (Machens et al., 2000; Grant et al., 2001; Park et al., 2009). Integra is a dermoconductive product, which stimulates migration of cells into the wound and stimulates the formation of a new dermis (Garwood and Steinberg, 2016). The composition of this material is similar to skin, consisting of a dermal matrix, which helps in the formation of blood vessels and granulation tissue, and a region composed of silicone, which acts as an epidermis. In human patients, Integra has been combined with NPWT (Park et al., 2009). It increases contact with the wound bed, improves graft take and minimizes complications such as the ingrowth of granulation tissue into the contact layer material. Good clinical results have been achieved in human patients after covering exposed bones, tendons and joints (Park et al., 2009).

Furthermore, the possibility of stem cell use in combination with NPWT has been studied. Epidermal, dermal and adipose-derived stem cells are interesting as they have a high level of plasticity (Orgill and Bayer, 2013). The combination of NPWT and mesenchymal stem cells has been studied in rats. The study showed that the vascularization of an acellular dermal matrix is accelerated and improved by combining NPWT and stem cells when compared to the separate use of these techniques (Sahin et al., 2014).

Currently, the effect of NPWT on the bacterial load of wounds remains controversial (Nolff et al., 2015c). Whereas some studies claim reduction of the bacterial burden (Morykwas et al., 1997; Li et al., 2015), many others state otherwise (Weed et al., 2004; Demaria et al., 2011). Instillation NPWT (NPWTi) is a recent therapy, involving the application of topical solutions or bioactive agents such as antiseptics or antibiotics onto the wound bed (Huang et al., 2014). It has proven suitable for contaminated and infected human and animal wounds (Allen et al., 2014; Nolff et al., 2015b; Nolff, 2016). Moreover, it has shown to improve granulation tissue production compared to traditional NPWT, to decrease or prevent bacterial proliferation, and avoid cross-contamination compared to conventional lavage therapy (Lehner et al., 2011; Allen et al., 2014). The current NPWT devices allow for programmed application in order to deliver an antiseptic solution at preset times and volumes. Moreover, continuous application is possible (Gupta et al., 2015; Wolvos, 2015). In comparison to conventional lavage therapy, NPWTi has shown to cause less tissue damage, and therefore less bacterial penetration and clinical infection occurring in the wound (Allen et al., 2014). So far, the application of NPWTi has only been reported twice in veterinary medicine: first as a case report (Nolff et al., 2015b) and recently as a case series (Nolff, 2016). The authors subjectively claimed an improved treatment outcome.

Another potential approach to deal with the bacterial load during NPWT is the concomitant use of interposing dressings that have anti-bacterial properties, such as honey (Rudzka-Nowak et al., 2010; Maguire et al., 2015), or silver (Mullally et al., 2010). Honey may interfere with the proliferation of bacteria, viruses, fungi and protozoa due to its specific characteristics, which are the hydrogen peroxide content (antimicrobial), low pH, hyperosmotic effect, the methylglyoxal content (antimicrobial) and its stimulation of the production of pro-inflammatory cytokines (Rudzka-Nowak et al., 2010; Maguire et al., 2015). Silver has an antimicrobial effect for it can damage the bacterial wall by interfering with DNA and RNA transcription (Mullally et al., 2010). In this way, both honey and silver may lower infection rates, prevent bacteremia and act against resistant microorganisms, such as *Staphylococcus aureus* and *Pseudomonas* (Woods et al., 2012; Balsa and Culp, 2015). Considering the beneficial properties of NPWT and an antimicrobial dressing, a combination of these applications has been suggested to be advantageous for wound treatment outcome (Mullally et al., 2010; Rudzka-Nowak et al., 2010). It should be kept in mind that confounding variables including the bacterial species, its virulence, the immune system of the patient and the debridement technique play a critical role in bacterial load and colonization (Moues et al., 2007; Li et al., 2015). For this reason, the construction of studies with a proper control group is questioned (Moues et al., 2007; Glass and Nanchahal, 2012).

The use of NPWT in preventive medicine has

great potential. Its application on postoperative surgical incisions have been described in humans as well as in animals (Hudson et al., 2015; Nolff and Meyer-Lindenberg, 2016). The beneficial effects include a decrease of edema, seroma and/or hematoma formation, and it may prevent surgical site infections and wound dehiscence (Horch, 2015; Nolff and Meyer-Lindenberg, 2016; Suh et al., 2016). There is only one published case report, which describes preventive incisional NPWT in a Rottweiler (Nolff et al., 2015a). More research with control groups is needed to explore the effects on closed incisions in veterinary medicine.

Lastly, NPWT is not limited to the treatment of wounds. In human patients, open abdomen management with NPWT has lowered morbidity and mortality (Coccolini et al., 2015; Nolff and Meyer-Lindenberg, 2016). Only two case series have been reported in veterinary medicine (Buote and Havig, 2012; Cioffi et al., 2012). In animal patients, open abdomen management is based on establishing drainage, controlling infection and stabilizing the patient with less focus on avoiding fascial retraction (loss of abdominal domain), which is an important issue in human patients (Cioffi et al., 2012; Nolff and Meyer-Lindenberg, 2016). Animal case series describe increased patient comfort, since it is a relatively simple way to manage an open abdomen: dressings may stay in place for several days and movement is enabled with a portable device. Postoperative patient management has also been improved due to the more hygienic management of effusions. Despite these positive findings, there are no studies yet, which compare to a control group (Nolff and Meyer-Lindenberg, 2016).

In conclusion, negative pressure for the treatment of wounds has been applied for centuries. In veterinary medicine however, it has found favor only recently. Positive and promising results have been documented, but more controlled studies are needed to clarify the exact mechanisms and beneficial effects of NPWT in animal patients. At last, establishing better guidelines and recommendations for each indication in human and veterinary medicine will improve the clinical use of this therapy.

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VAN ALLE TIJDEN: SLECHTE BETALERS

Pieter Milliau, een van de eerste Vlaamse dierenartsen gediplomeerd aan de nieuwe veeartsenijschool van Alfort (Parijs), kreeg in 1790 al te maken met slechte betalers. De ‘peerdemeester’ spande een zaak in voor de rechtbank die de beschuldigde, Joannes Colle voerman en buurman van Milliau in de Gentse Dampoortststraat (Jooremaykensmeersch) veroordeelde tot het betalen van de verschuldigde 4 pond - 3 schellingen - 6 groten ‘ (ongeveer 20 bouwvakkersdaglonen uit die tijd) voor het ‘cureren van (de) ‘peerden ende leveringen van medecynen ende salven’. De heren rechters namen (toen al) hun tijd. De uitspraak volgde precies twee jaar na de aanklacht.
Bron: Bibliotheek UGent, Ephemera, chevaux

Luc Devriese

Pathofysiologie van lipopolysaccharide geïnduceerde inflammatoire respons bij vogels

Pathophysiology of lipopolysaccharide-induced inflammatory response in birds

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SAMENVATTING

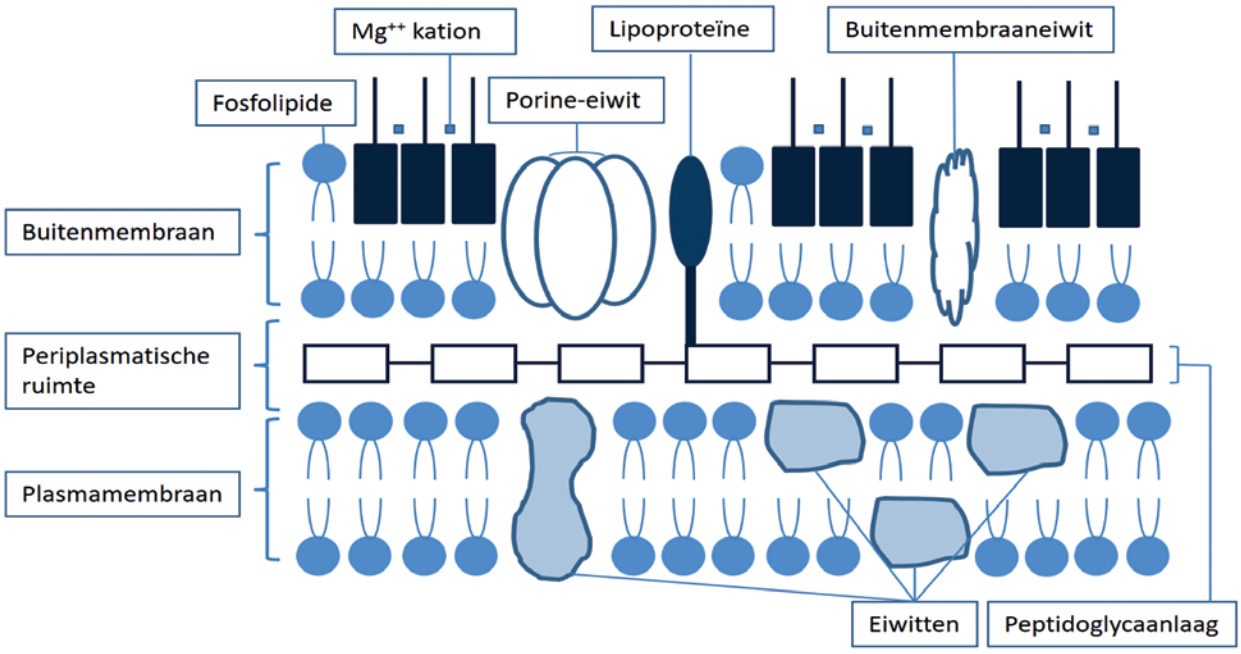
Inflammatie is een beschermende respons op infectie en/of weefselschade die gepaard gaat met de migratie van immuuncellen en mediatoren van de circulatie naar het betreffende weefsel. Deze respons dient om de initiële noxe (onder andere lipopolysaccharide of LPS) te verwijderen en genezing en herstel van het beschadigde weefsel te bekomen. LPS is een onderdeel van de buitenste celmembraan van gramnegatieve bacteriën dat pro-inflammatoire eigenschappen heeft en na toediening bij vogels een ontstekingsreactie teweegbrengt. Deze ontstekingsreactie gaat gepaard met onder andere veranderingen in lichaamstemperatuur, de productie van pro-inflammatoire cytokinen en vorming van acutefase-eiwitten, leukocytose en ziektegedrag. In welke mate elk van deze symptomen aanwezig is bij vogels hangt af van de vogelsoort. Bovendien zijn er verschillen met zoogdieren. De karakteristieken en pathofysiologische gevolgen van een ontstekingsreactie worden vaak bestudeerd in LPS-inflammatiemodellen. Deze inflammatiemodellen kunnen vervolgens toegepast worden in farmacodynamiekstudies om het klinisch effect van anti-inflammatoire geneesmiddelen, zoals niet-steroïdale ontstekingsremmers (NSAIDs) te beoordelen. In dit artikel wordt een overzicht gegeven van de LPS-geïnduceerde inflammatoire respons bij vogels.

ABSTRACT

Inflammation is a protective response to infection and/or tissue damage and it induces migration of immune cells and mediators of immune response from the circulation to the infected and/or damaged tissue. This response will remove the initial noxe (e.g. lipopolysaccharide or LPS) and tissue healing will be stimulated. LPS is part of the outer membrane of gram-negative bacteria and causes an inflammatory response in birds due to its proinflammatory properties. As a result to this inflammatory response, birds develop a change in body temperature, increased production of proinflammatory cytokines and acute phase proteins, show leukocytosis and sickness behavior. The magnitude of these symptoms in birds depends on the bird species and differs from the symptoms in mammals. The characteristics and pathophysiology of an inflammatory response are frequently studied using LPS inflammation models. These models can further be applied for pharmacodynamic studies to assess the clinical effect of different anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs or NSAIDs. In this paper, an overview of the LPS-induced inflammatory response in birds is given.

INLEIDING

Gramnegatieve bacteriën, zoals *Escherichia coli* (*E. coli*) en *Salmonella spp.*, bestaan uit een cytoplasma omgeven door een membraan die opgebouwd is uit een buitenmembraan, een plasmamembraan en ertussen een peptidoglycaanlaag die zorgt voor de stevigheid van de cel (Figuur 1). De buitenmembraan bevat twee lagen, waarvan de binnenste laag bestaat uit glycerofosfolipiden en de buitenste laag voornamelijk uit lipopolysacchariden (LPS) en enkele fosfolipiden (Wright en Kangeasaki, 1971). Daarnaast bevat de buitenmembraan lipoproteïnen, porineproteinen en membraanproteïnen die verankerd zijn in de onderliggende peptidoglycaanlaag. Deze membraan speelt een rol in het transport van nutriënten vanuit



Figuur 1. Schematische illustratie van de buitenmembraan, celwand en peptidoglycaanlaag van een gramnegatieve bacterie (naar Todar, 2006).

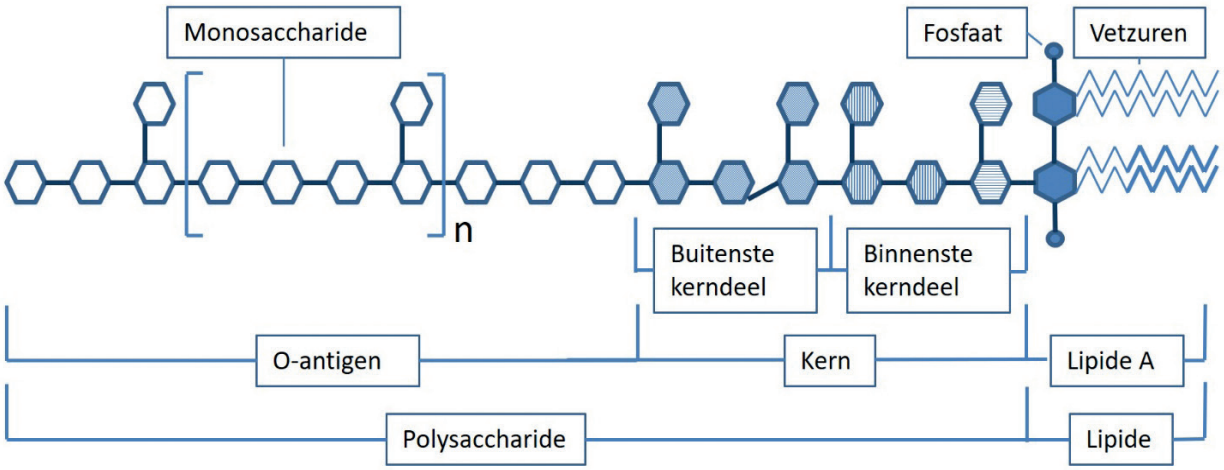
de omgeving naar de bacterie en de fysiologische en pathofysiologische interacties tussen bacterie en gastheercellen. Tevens kan deze membraan de intrede van toxische stoffen inhiberen, zoals antimicrobiële middelen (Rietschel et al., 1994; Holst, 2007). De peptidoglycaanlaag is opgebouwd uit verschillende ketens bestaande uit afwisselend N-acetylglucosamine- en N-acetylmuraminezuur-moleculen die onderling worden verbonden via tetrapeptiden. Deze tetrapeptiden zijn opgebouwd uit L-alanine, D-glutamaat, diaminopimelinezuur en D-alanine. De plasmamembraan bestaat uit fosfolipiden en proteïnen (Todar, 2006).

LPS is een endotoxine dat een sterke immuunrespons kan veroorzaken bij mens en dier. Het is een glycolipide dat bestaat uit drie covalent verbonden delen: het lipide A, een kern met polysacchariden en het O-antigeen (Figuur 2). De LPS-molecule is verankerd in de buitenmembraan van de gramnegatieve bacterie via het lipide A-domein (Raetz et al., 1988; Rietschel et al., 1993; Rietschel et al., 1994; Holst, 2007) (Figuur 2). Lipide A is een lipide dat bestaat uit verzadigde vetzuren en gefosforyleerd N-acetylglucosamine (Lüderitz et al., 1982; Todar, 2006). Dit lipide vormt het hydrofobe domein van de LPS-molecule en wekt een immuunrespons op bij de gastheer (Holst, 2007). Het kerngedeelte is een polysaccharide dat bestaat uit een korte suikerketen, die onder andere heptose en 2-keto-3-deoxyoctoninezuur bevat en het lipide A en O-antigeen verbindt. Het O-antigeen is een polysaccharide dat bestaat uit herhalende subeenheden van oligosacchariden. Het vormt het hydrofiele domein van de LPS-molecule en heeft een sterk antigene werking (Rietschel et al., 1994; Todar, 2006).

LPS heeft pro-inflammatoire eigenschappen en brengt een ontstekingsreactie op gang die gepaard gaat met immunologische veranderingen (Skarnes et al., 1981; Morimoto et al., 1987; Haudek et al., 2002). Inflammatie is een beschermende respons op infectie en/of weefselschade die gepaard gaat met de migratie van immuuncellen en mediatoren naar het betreffende weefsel. Deze respons dient om de initiële noxe te verwijderen en genezing en herstel van het beschadigde weefsel te bekomen (Kumar et al., 2015, Lees et al., 1991). In dit artikel wordt een overzicht gegeven van de LPS-geïnduceerde inflammatoire respons bij vogels.

INFLAMMATOIRE RESPONS OP LPS

Bij de LPS-geïnduceerde ontstekingsreactie is er interactie tussen het lipide A-domein van de LPS-molecule en “LPS binding protein” (LBP). LBP is een serumglycoproteïne dat wordt geproduceerd door hepatocyten en in verhoogde concentratie aanwezig is tijdens de ontstekingsreactie (Tobias et al., 1986; Ramadori et al., 1990; Schumann et al., 1990). Deze interactie leidt tot activatie van gastheercellen, waaronder mononucleaire cellen, polymorfonucleaire cellen, endotheelcellen en trombocyten (Rietschel et al., 1994). LBP bindt aan LPS en interageert met “cluster of differentiation 14” (CD14), dat bestaat in een oplosbare vorm en een membraangebonden vorm die aanwezig is op het celoppervlak van mononucleaire cellen. Het gevormde CD14:LPS complex zorgt in membraangebonden vorm voor de activatie van de betreffende cel en in oplosbare vorm voor de activatie van cellen die geen membraangebonden CD14 bevat-

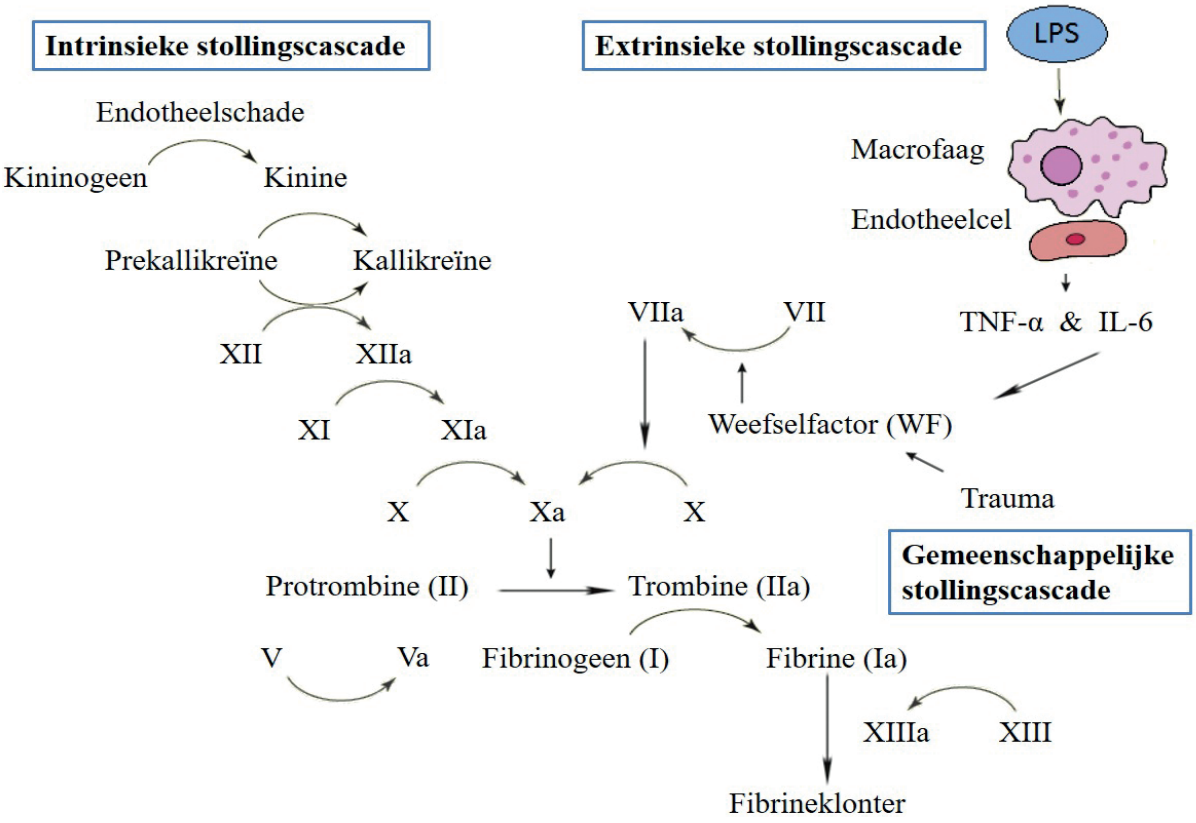


Figuur 2. Schematische voorstelling van de structuur van LPS (naar Tobias et al., 1999).

ten (Mayeux, 1997; Moore en Barton, 1998; Sass et al., 2002; Jerala, 2007). Deze activatie wordt gemedieerd door de interactie tussen CD14 en "toll-like receptor 4" (TLR-4) en leidt zo tot de activatie van "nuclear factor κB" (NFκB) (Dil en Qureschi, 2002; Doyle en O'Neill, 2006). De activatie van heterofielen door LPS gebeurt bij kippen eveneens door middel van LBP, CD14 en TLR-4 (Kogut et al., 2005). CD14, dat bij zoogdieren een glycosylfosfatidylinositol-geankerd eiwit is, is bij kippen echter een transmembranair eiwit dat minder mobiel is in de celmembraan. Hierdoor reageren kippen minder sterk op LPS-toediening dan zoogdieren (De Boever et al., 2009; Wu et al., 2009). NFκB fungeert als transcriptiefactor voor genen die coderen voor pro-inflammatoire cytokinen, zoals "tumor necrosis factor α" (TNF-α), interleukine 1 (IL-1), interleukine 6 (IL-6) en interferon γ (IFN- γ) (Heinrich et al., 1990; Baeuerle en Henkel, 1994). TNF-α wordt vrijgesteld door onder andere monocyten, macrofagen, T-cellen en B-cellen en stimuleert de productie van IL-1β en IL-6 (Witkamp en Monshouwer, 2000). IL-1, IL-6 en TNF-α zorgen onder andere voor koortsinductie, verhoogde synthese van acutefase-eiwitten in de lever, differentiatie van immuuncellen, stijging van het serumgehalte van cortisol, cortisone en bij vogels corticosterone en de activatie van het vasculaire endotheel (Durum et al., 1990; Schneider et al., 2001). IFN-γ, IL-1β en IL-10 zijn aangetoond bij de kip (Jakowlew et al., 1988; Jakowlew et al., 1990; Digby en Lowenthal, 1995) en hebben een gelijkaardige functie als bij zoogdieren (Lillehoj et al., 1992; Song et al., 1997; Weining et al., 1998; Rothwell et al., 2004). In een in-vitrostudie werd de productie van TNF-α aangetoond bij macrofagen afkomstig van kippen geïnfecteerd met *Eimeria maxima* en *E. tenella* (Byrnes et al., 1992). Daarnaast werd bij de kip ook de aanwezigheid aangetoond van de TNF-α receptor en TNF-like ligand 1A (TL1A), wat een gelijkaardige activiteit heeft als TNF-α (Rautenschlein et al., 1999; Koskela et al., 2004; Schneider et al., 2004; Goetz et al., 2004; Kaiser et al., 2005; Hong et al., 2006; De Boever et al., 2009). TL1A wordt onder andere geproduceerd door lymfocyten in de milt, lever en het darmepitheel en stimuleert de synthese van IL-1β, IL-6 en IFN-γ. Daarnaast zorgt het voor een verhoogde vrijstelling van induceerbaar stikstofoxidesynthase (iNOS) en cyclooxygenase 2 (COX-2) (Geller et al., 1993; Park et al., 2007; Takimoto et al., 2008). De verhoogde productie van iNOS zorgt voor de ontwikkeling van hypotensie (De Boever et al., 2009). De ontstekingsreactie gaat eveneens gepaard met hypocholesterolemie als gevolg van een verandering in het cholesterol- en lipoproteïnemetabolisme ter hoogte van de lever (Kushner, 1982). De belangrijkste kenmerken van de ontstekingsreactie zijn verandering in lichaamstemperatuur, de productie van pro-inflammatoire cytokinen en hepatische acutefase-eiwitten, leukocytose, activatie van de hypothalamo-hypofysaire as, onderdrukking van de hypothalamo-hypofyso-gonadale as en het optreden van ziektegedrag (Klasing, 2004; Owen-Ashley en Wingfield, 2007). In Tabel 1 wordt een overzicht gegeven van de gepubliceerde in-vivo-en -in-vitro-LPS-inflammatiemodellen bij vogels.

Veranderingen in lichaamstemperatuur

Koorts is het gevolg van de cascade die in gang wordt gezet door exogene pyrogenen, zoals LPS, die leukocyten aanzetten tot een verhoogde vrijstelling van IL-1β en TL1A. Dit zijn endogene pyrogenen die onder andere zorgen voor een verhoogde expressie van COX en de synthese van prostaglandine E_2 (PGE_2) (Heinrich et al., 1990; Kumar et al., 2015). PGE_2 zorgt ter hoogte van de hypothalamus voor een verhoogde productie van neurotransmitters die de setpoint voor lichaamstemperatuur verhogen (Netea et al., 2000). Een verhoogde lichaamstemperatuur sti-



Figuur 3. Factoren betrokken bij de intrinsieke en extrinsieke stollingscascade en hun rol bij de vorming van een bloedklonter (naar Karima et al., 1999).

muleert de synthese van acutefase-eiwitten. Daarnaast stimuleert het de activiteit van heterofielen en macrofagen, zorgt het voor ijzersequestratie en inhibeert het de bacteriële groei (Netea et al., 2000). Het effect van pyrogenen op de lichaamstemperatuur is afhankelijk van de balans tussen productie en verlies van warmte, die beide beïnvloed worden door de lichaamsgrootte en omgevingstemperatuur (Kluger, 1986). Na experimentele toediening van LPS werd er in een studie bij zebravinken (*Taeniopygia guttata*) en andere Passeriformen hypothermie vastgesteld (Burness et al., 2010), en bij kippen, duiven, eenden en Japanse kwartels (*Coturnix japonica*) hyperthermie (Baert et al., 2005a; Owen-Ashley et al., 2006; De Boever et al., 2010). Deze hyperthermie wordt bij de kippen en duiven voorafgegaan door een korte periode van hypothermie. Bij herhaalde toediening van LPS bij kippen blijft hypothermie zelfs volledig uit (De Boever et al., 2008). De sterk uitgesproken hypothermie die optreedt bij Passeriformen wordt veroorzaakt door een hoge lichaamsoppervlakte-volumeratio, waardoor er relatief meer warmteverlies optreedt dan bij grotere vogels en de thermoregulatie moeilijker verloopt (Owen-Ashley en Wingfield, 2007). Daarnaast is de gemiddelde lichaamstemperatuur van vogels relatief hoog (41°C), waardoor elke bijkomende temperatuurverhoging door een verhoogd metabolisme moeilijk is, aangezien het metabolisme voor elke stijging van de lichaamstemperatuur met 1°C moet verhogen met 10% (Kluger, 1979; Owen-Ashley en Wingfield, 2007). Knaagdieren met dezelfde lichaamsoppervlakte-volumeratio en een lagere gemiddelde lichaamstemperatuur (36-37°C) zijn wel in staat om hyperthermie te ontwikkelen tijdens de ontstekingsreactie (Owen-Ashley en Wingfield, 2007).

Verschillende parameters hebben een invloed op de lichaamstemperatuur van vogels tijdens de ontstekingsreactie, zoals de leeftijd en het moment van de dag. Zo treedt er bij zebravinken hypothermie op na injectie van LPS gedurende de dag en hyperthermie na injectie van LPS gedurende de nacht. Dit kan verklaard worden door het feit dat de lichaamstemperatuur van deze vogels overdag hoger is en tevens toeneemt door warmteproductie tijdens activiteit, waardoor er een risico bestaat op oververhitting indien de vogels hyperthermie zouden ontwikkelen (Sköld-Chiriac et al., 2015). Bij oververhitting wordt de lichaamstemperatuur dermate hoog dat er celnecrose, oedeem en mogelijk bloedingen optreden ter hoogte van onder andere de lever, het centrale zenuwstelsel, de nieren en de hartspier (Malamud et al., 1946; Gore en Isaacson, 1949; Fajardo, 1984). 's Nachts is de li-

chaamstemperatuur van vogels lager, waardoor het risico op oververhitting lager is en bijgevolg hyperthermie ontwikkeld kan worden om infecties te bestrijden (Sköld-Chiriac et al., 2015). Ook de leeftijd van vogels beïnvloedt de verandering in lichaamstemperatuur tijdens de acutefaserespons. Bij kippen van één tot twee weken oud treedt er hypothermie op gevolgd door hyperthermie, terwijl kippen van drie tot acht weken oud direct hyperthermie ontwikkelen (Jones et al., 1983). Dit kan verklaard worden door de beperkte aanwezigheid van het thermoregulatievermogen bij zeer jonge kippen, waardoor hyperthermie minder efficiënt geïnduceerd wordt (Jones et al., 1983; Frafield en Kaplanski, 1998). Deze bevinding verschilt van die van De Boever et al. (2009) waarbij LPS-geïnduceerde hyperthermie bij kippen van drie en vijf weken oud wel werd voorafgegaan door een korte periode van hypothermie. Dantonio et al. (2016) toonden aan dat stikstofoxide (NO) eveneens een rol speelt in het mediëren van de lichaamstemperatuur tijdens de ontstekingsreactie bij vogels. Intramusculaire toediening van een NO-synthase-inhibitor (N-nitro-L-arginine methylester of L-NAME) aan vleeskippen van vijf dagen oud, die intramusculair LPS toegediend gekregen hadden, onderdrukte de thermogenese bij een omgevingstemperatuur die lager was dan de thermoneutrale zone voor deze dieren (35-36°C). Dit leidde tot een versterkte hypothermiefase en een minder uitgesproken hyperthermiefase bij deze dieren. Bij een thermoneutrale omgevingstemperatuur werd de lichaamstemperatuur niet door L-NAME beïnvloed, wat aantoont dat NO perifeer inwerkt op de thermogenese en de centrale temperatuurregulatie niet medieert (Dantonio et al., 2016). Dit werd ook aangetoond bij ratten (Steiner et al., 2004). Het feit dat de PGE_2-concentratie in de hersenen evenmin wordt beïnvloed door L-NAME-injectie bevestigt deze theorie. Bij ratten stimuleert NO de thermogenese ter hoogte van het bruine vetweefsel door middel van vasodilatatie. Bij vogels is er geen bruin vetweefsel aanwezig, maar zou thermogenese worden bekomen via bepaalde mechanismen in de skeletspieren (Kluger, 1991; Nagashima et al., 1994; Steiner et al., 2001; Branco et al., 2014). Om na te gaan welke mechanismen dit precies zijn, is verder onderzoek noodzakelijk (Dantonio et al., 2016).

Pro-inflammatoire cytokinen en acutefase-eiwitten

In Tabel 1 wordt weergegeven welke cytokinen onderzocht werden in de beschikbare in-vitro-en -in-vivo-LPS-modellen bij vogels (mRNA-expressie en/of eiwitgehalte). Er wordt eveneens vermeld wanneer de maximale of piekconcentratie bereikt werd. De productie van pro-inflammatoire cytokinen leidt tevens tot een verhoogde productie van acutefase-eiwitten in de hepatocyten (Heinrich et al., 1990). Welke acutefase-eiwitten aanwezig zijn, is diersoortafhankelijk (Heinrich et al., 1990). De belangrijkste acutefase-eiwitten die in concentratie toenemen tijdens een inflammatoire respons bij de kip zijn hemopexine, α1-zure glycoproteïne (α1-AG), ceruloplasmine, transferrine, fibronectine en serum amyloid A (Curtis en Butler, 1980; Nakamura et al., 1998; Takahashi et al., 1998; Chamanza et al., 1999; Barnes et al., 2002). Ceruloplasmine oxideert tweewaardig ijzer (Fe^{2+}) tot driewaardig ijzer (Fe^{3+}) en faciliteert zo het transport van ijzer door transferrine (Murata et al., 2004). Transferrine wordt geproduceerd als respons op de verhoogde vrijstelling van IL-6 (Xie et al., 2002). Het bindt ijzerionen (Fe^{3+}), zodat er minder ijzerionen beschikbaar zijn voor pathogenen en het moduleert de functies van macrofagen en heterofielen (Xie et al., 2002; Murata et al., 2004). De verhoogde productie van acutefase-eiwitten in de lever is het gevolg van een verhoogde activiteit van specifieke enzymen in de hepatocyten (Kushner et al., 1982). Daarnaast zorgen onder andere de toediening van LPS en het gewichtsverlies voor een verhoogd levermetabolisme. Dit heeft als gevolg dat het gewicht van de lever toeneemt (Koh, 1996; Sherwin en Sobenes, 1996; Bayyari et al., 1997; Xie et al., 2000).

Leukocytose

Leukocytose is het gevolg van een verhoogde vrijstelling van heterofielen uit de reservepool van het beenmerg veroorzaakt door de vrijstelling van "granulocyt-colony stimulating factor" (G-CSF) en granulocyt-macrofaag-CSF (GM-CSF) door macrofagen (Morrison en Ulevitch, 1978; Kogut et al., 1997; Kaiser et al., 2005). Deze heterofilie wordt bij kalkoenen voorafgegaan door een korte periode van leukopenie als gevolg van een daling van het aantal heterofielen in de circulatie door sequestratie in de bloedvaten van de longen en lever (Harmon, 1998; De Boever et al., 2009). Bij de kip werd eveneens leukopenie aangetoond die behalve door sequestratie van heterofielen in de longen ook het gevolg zou zijn van apoptose van de circulerende heterofielen (De Boever et al., 2009).

Ziektegedrag

Naast een verandering in lichaamstemperatuur, de productie van pro-inflammatoire cytokinen en acutefase-eiwitten en leukocytose zorgt de ontstekingsreactie voor gedragsveranderingen. Deze omvatten onder andere een verminderde activiteit en een verminderde voeder- en drinkwateropname met gewichtsverlies als gevolg. Het doel van deze gedragsveranderingen is om de energie die gebruikt wordt voor activiteiten die niet direct essentieel zijn voor overleving, zoals foerageren, groei en reproductie, te verminderen en de aanvoer van belangrijke nutriënten, zoals zink en ijzer voor de replicatie van bacteriën te beperken (Hart, 1988; Klasing, 1984; Langhans, 2000). Als de voedselopname echter te beperkt is, daalt het lichaamsgewicht sterk en kunnen er ziekte en cachexie optreden, waardoor de kans op overleving en herstel kleiner is (Plata-Salamán, 1996;

Tabel 1. Overzicht van in-vitro en in-vivo-LPS-inflammatiemodellen bij vogels.

LPS (sero)type	Dosering	Vogelsoort/ cel type	Leef-tijd	Gewicht (g)	Toe-diening	Lichaams-temperatuur	Cytokinen	Referentie
E. coli O127:B8	1 mg/dier	vleeskip	3w	700 -900	IP	↑ (piek 3u n.t.)	-	(Fraifeld et al., 1995)
E. coli O127:B8	0,9 mg/dier	legkip		-	IP	-	-	(Inoue et al., 1997)
E. coli O127:B8	1 mg/kg LG	vleeskip	5w	1400	IV	↑ (piek 2u n.t.) bifasisch (1-5u/ 6-8u)	-	(Baert et al., 2005a,b)
E. coli O127:B8	1 mg/kg LG	vleeskip	5w	-	IV	↑ bifasisch (3-5u / 9-12u)	-	(Jones et al., 1981)
E. coli O127:B8	1 mg/kg LG	vleeskip	3w	-	IV	↓ (piek 1u n.t.) gevolgd door ↑(piek 8u n.t.)	↑ IL-6 plasma	(De Boever et al., 2008)
			5w	-	IV	↓ gevolgd door ↑	-	
E. coli O127:B8	2,5 mg/kg LG	vleeskip	5w	-	IV	↓ (piek 3u n.t.) gevolgd door ↑(piek 12u n.t.)	↑ IL-6 plasma ↑ IL-1β en IL-6-expressie in heterofielen	(De Boever et al., 2009)
E. coli O127:B8	2,5 mg/kg LG	vleeskip	5w	1270	IV	↑ (piek 12u n.t.)	↑ IL-6 plasma ↑ IL-1β en IL-6-expressie in heterofielen	(De Boever et al., 2010)
E. coli O128:B12	2,5 mg/dier	dubbeldoel	5w	500 - 650	IP	↑ (piek 2u n.t.)	-	(Johnson et al., 1993)
E. coli O55:B5	1 mg/kg LG	legkip		1500 - 2000	IP	-	-	(Barnes et al., 2002)
E. coli O55:B6	3 mg/kg LG	vleeskip	3w	-	IP	-	↑ IL-6 plasma	(Shen et al., 2010)
E.coli O111:B4	4 µg/mL	macrofagen kip		-	in vitro	-	↑TNF-α (4u n.t.) gevolgd door↓(18-48u n.t.)	(Hong et al., 2006)
E. coli	0,0015 mg/kg LG	vleeskip	-	-	IV ICV	↑ ↑	- -	(Macari et al., 1993)
E. coli	0,001 mg/kg LG 0,010 mg/kg LG 0,1 mg/kg LG	pekingeend	-	2400 - 3400	IV	↑ (piek 3u n.t.) ↑ (piek 4u n.t.) ↑ (piek 4,9u n.t.)	- - -	(Maloney en Gray, 1998)
E. coli	2 mg/kg LG	legkip	-	120	IC	-	↑ IL-6 serum	(Nakamura et al., 1998)
E. coli	0,3 mg/dier	vleeskip	4w	-	SC	-	-	(Buyse et al., 2007)
Salmonella Typhimurium	0,5 mg/kg LG	Japanse kwartel	18d	55	IP	-	-	(Koutsos en Klasing, 2001)
	1 mg/kg LG		18d	55	IP	↓ (piek 1u n.t.) gevolgd door ↑(piek 10u n.t.)	-	
	2,5 mg/kg LG		18d	55	IP	↓ (piek 1u n.t.) gevolgd door ↑(piek 10u n.t.)	-	
	2,5 mg/kg LG		23d	72	IP	↑ (piek 10u n.t.)	-	
	7,5 mg/kg LG		23d	72	IP	↑ (piek 10u n.t.)	-	
	22,5 mg/kg LG		23d	72	IP	↑ (piek 10u n.t.)	-	
	7,5 mg/kg LG (3x)		10d	24	IP	↑ (piek 5u n.t.)	↑ mRNA-expressie IL-1β lever en milt	(De Boever et al., 2008)
Salmonella Typhimurium	4 µg/mL	macrofagen kip	-	-	in vitro	-	↑TNF-α (4u n.t.) gevolgd door↓(18-48u n.t.)	(Hong et al., 2006)
Salmonella Typhimurium	5 mg/kg LG	vleeskip	3w	690	IV	-	↑ IL-6 plasma	(Xie et al., 2000)
Salmonella Typhimurium	0,1 - 5 mg/kg LG	legkip	34d	327	IP	↑ (piek 4u n.t.)	↑ mRNA-expressie IL-1β en IFN-γ milt	(Leshchinsky en Klasing, 2001)
Salmonella Typhimurium	5 mg/kg LG	vleeskip	34d	1194	IP	↑ (piek 12u n.t.)	↑ mRNA-expressie IL-1β en IFN-γ milt	(Leshchinsky en Klasing, 2001)
Salmonella Typhimurium	0,0005 mg/dier	vleeskip	4w	-	IV	-	↑ mRNA-expressie IL-1β, IFN-γ en MGF milt	(Leshchinsky en Klasing, 2003)
Salmonella Enteritidis	4 µg/mL	macrofagen kip	-	-	in vitro	-	↓TNF-α (18u n.t.)	(Hong et al., 2006)
Salmonella Minnesota	10 µg/mL	trombocyten vleeskip	-	-	in vitro	-	↑ mRNA-expressie IL-1β, IL-6, IL-12	(Ferdous et al., 2008)
Salmonella typhosa	0,1 mg/kg LG	pekingeend	-	2400 - 3200	IM	↑ (piek 6u n.t.)		(Gray et al., 2005)

(IP: intraperitoneaal, IV: intraveneus, ICV: intracerebroventriculair, SC: subcutaan, IM: intramusculair, IL-1β: interleukine-1β, IL-6: interleukine-6, IL-12: interleukine-12, TNF-α: tumor necrosis factor α, IFN-γ: interferon-γ, MGF: myelomonocytic growth factor, n.t.: na toediening)(- = niet onderzocht)

Kyriazakas et al., 1998). De expressie van ziektegedrag wordt beïnvloed door verschillende factoren, zoals omgevingstemperatuur en seizoen. Zo is de vermindering van voedselopname meer uitgesproken bij vogels gehuisvest bij een thermoneutrale omgevingstemperatuur (34°C) dan bij vogels gehuisvest bij een lagere omgevingstemperatuur (15°C) (Burness et al., 2010). De gedragsveranderingen bij de mannelijke witkruingors (*Zonotrichia leucophrys*) in gevangenschap zijn meer uitgesproken tijdens de fokperiode dan in de winter. Dit kan verklaard worden door de grotere vetmassa tijdens de fokperiode met als gevolg een grotere energiereserve voor de ontstekingsreactie dan tijdens de winterperiode. Deze seizoensgebonden variatie in gedragsveranderingen komt niet voor bij vrouwelijke witkruingorsen (Owen-Ashley en Wingfield, 2007). Dit verschilt echter van het ziektegedrag van de wildlevende, mannelijke zanggors (*Melospiza melodia morphna*) die juist tijdens de winterperiode een grotere vetmassa heeft en bijgevolg duidelijkere gedragsveranderingen ondergaat als onderdeel van de ontstekingsreactie dan mannelijke dieren tijdens de fokperiode (Owen-Ashley en Wingfield, 2006). Daarnaast is het basale corticosterongehalte van mannelijke vogels tijdens de fokperiode hoger dan bij deze tijdens de winterperiode wegens een hogere energiebehoefte voor territoriaal gedrag, gonadale ontwikkeling en testosteronproductie (Ketterson et al., 1991; Ketterson en Nolan Jr., 1999). Hierdoor is er minder energie beschikbaar voor de ontstekingsreactie, zodat de vogels minder gevoelig zijn voor LPS (Owen-Ashley en Wingfield, 2006). Een hoog testosterongehalte zorgt eveneens voor een onderdrukking van de ontstekingsreactie (Wingfield, 1994).

LPS-GEÏNDUCEERDE SHOCK

De LPS-geïnduceerde ontstekingsreactie kan bij hoge dosering leiden tot endotoxine-shock of multipel orgaanfalen als gevolg van een overmatige productie van pro-inflammatoire mediatoren (onder andere TNF-α, IL-1β en NO). NO zorgt voor vasodilatatie, door middel van relaxatie van vasculair glad spierweefsel en een verminderde gevoeligheid voor vasoconstrictoren. Gecombineerd met een verminderde myocardfunctie zorgt dit voor hypotensie met mogelijk shock tot gevolg. Hypotensie gaat gepaard met hypoperfusie van de weefsels, waardoor hypoxie en weefselschade kunnen optreden (Karima et al., 1999). De interactie tussen NO en het superoxide anion (O_2^-) leidt tot de vorming van cytotoxische zuurstofradicalen (onder andere peroxynitriet of $ONOO^-$ en het hydroxylradicaal of OH^-) die eveneens weefselschade veroorzaken (Beckman en Koppenol, 1996). Naast weefselschade zorgen zuurstofradicalen voor de activatie van NF-κB, dat onder andere instaat voor de activatie van de productie van pro-inflammatoire cytokinen, waardoor de ontstekingsreactie verder wordt versterkt (Sen en Packer, 1996; Hierholzer et al., 1998; Karima et al., 1999). Adhesie van leukocyten aan endotheel gaat gepaard met beschadiging van de microvasculatuur, waardoor weefseloedeem optreedt en de weefselperfusie verder daalt (Golenbock et al., 1991; White et al., 1997; Chow et al, 1999; Karima et al., 1999; Lien et al., 2000). Neutrofielen zorgen na migratie door de endotheelcellaag voor de vrijstelling van zuurstofradicalen, maar stellen daarnaast ook verschillende proteasen vrij, zoals serineproteasen. Neutrofiel-elastase is een serineprotease dat zorgt voor de afbraak van verschillende eiwitten, waaronder transporteiwitten, membraaneiwitten, celreceptoren, fibronectine en collageen, maar ook fibrinolysefactoren, die een rol spelen in de afbraak van bloedklonters. De verhoogde expressie van weefselfactor en factor VIIa na LPS-toediening zorgt voor een verhoogde activatie van de stollingscascade (Levi et al., 1994; 1997) (Figuur 3). De combinatie van verhoogde stolling en verminderde fibrinolyse leidt tot de ontwikkeling van gedissemineerde intravasculaire stolling (DIC) (Karima et al., 1999). DIC is een verstoring van de hemostasebalans met verhoogde activatie van de stollingscascade en gaat gepaard met microvasculaire trombose en uitputting van stollingsfactoren, waardoor uiteindelijk bloedingen optreden met risico op multipel orgaanfalen (Johnson et al., 1998). Bepaalde stollingsfactoren, zoals trombine en factor Xa, stimuleren de vrijstelling van pro-inflammatoire cytokinen door endotheelcellen en monocyten en versterken de ontstekingsreactie (Karima et al., 1999). Dit mechanisme, zoals het verloopt bij zoogdieren, is bij vogels nog niet volledig opgehelderd.

Vogels zijn minder gevoelig voor de ontwikkeling van endotoxine-shock dan zoogdieren. Dit is het gevolg van verschillen in het stollingsmechanisme en het activatiemechanisme van interferon β (IFN-β). Het stollingsmechanisme van zoogdieren bestaat uit een intrinsieke en extrinsieke cascade die beide nodig zijn voor de vorming van trombine dat de gemeenschappelijke stollingscascade activeert tot de vorming van de uiteindelijke bloedklonter (Figuur 3). De intrinsieke stollingscascade is bij kippen en struisvogels zwak vergeleken met zoogdieren en bepaalde onderdelen van de extrinsieke stollingscascade, zoals stollingsfactor VII, zijn beperkt tot niet aanwezig. Hierdoor verloopt de stollingscascade trager bij vogels dan bij zoogdieren (Frost et al., 1999). Stollingsfactor X, die een sleutelrol speelt in de vorming van trombine, is bij kippen echter wel aanwezig (Stopforth, 1970) en werd eveneens bij struisvogels aangetoond (Frost et al., 1999). Fibrinolyse gebeurt bij vogels even efficiënt als bij zoogdieren (Frost et al., 1999). Deze verschillen met zoogdieren kunnen, door een kleinere kans op DIC, verklaren waarom vogels resistenter zijn tegen de hemodynamische LPS dan zoogdieren (Adler en DaMassa, 1979).

IFN-β zorgt voor de productie van chemokinen en adhesiemoleculen die een rol spelen bij inflam-

matie (Shimizu et al., 1990; Sikorski et al., 2011). Verschillen in het activatiemechanisme van IFN-β tussen zoogdieren en vogels uiten zich in de afwezigheid van de signaaltransductie tussen TLR4, Toll/IL-1 receptor-domein bevattend adaptoreiwit (TRIF) en TRIF-gerelateerde adaptoreiwit (TRAM) na binding met LPS bij vogels (Berczi et al., 1966; Keestra en Van Putten, 2008). De afwezigheid van IFN-β zorgt bij muizen voor resistentie tegenover LPS-geïnduceerde endotoxine shock (Karaghiosoff et al., 2003). Bij vissen, die een hoge resistentie hebben tegenover endotoxine-shock, is deze signaaltransductie eveneens afwezig (Iliev et al., 2005).

CONCLUSIE

Door middel van LPS-inflammatiemodellen wordt er bij verschillende diersoorten een ontstekingsreactie opgewekt die gepaard gaat met veranderingen op cellulair niveau, fysiologische en gedragsveranderingen. De veranderingen die optreden bij vogels verschillen van deze bij zoogdieren. Zo speelt TNF-α bij zoogdieren een belangrijke rol bij inflammatie, terwijl bij vogels vooral TL1A van belang is en TNF-α enkel nog maar in vitro werd aangetoond. Daarnaast treedt er bij zoogdieren, zoals de hond, de muis, de rat, de cavia en de kat, onafhankelijk van de diersoort, koorts op na intraveneuze toediening van LPS (LeMay et al., 1990; Long et al., 1990; Kozak et al, 1994; Roth et al., 2002; McCann et al., 2005), terwijl dit niet altijd het geval is bij vogels. De gevoeligheid voor LPS verschilt eveneens tussen zoogdieren en vogels. Zo is er een veel hogere dosis LPS nodig om een ontstekingsreactie op te wekken bij kippen (1 mg/kg lichaamsgewicht of LG) dan bij kalveren (0,5 µg/kg LG) en varkens (15 µg/kg LG) (De Boever et al., 2008; Wyns et al., 2014; Plessers et al., 2015). Deze verschillen zijn onder andere het gevolg van een mobiliteitsverschil van CD14, waarbij dit eiwit minder mobiel is in de celmembraan van kippen dan in die van zoogdieren. Bijgevolg kan er minder activatie van de cel optreden na vorming van het CD14:LPS-complex. Tevens is er een sterk verschil in de lichaamsbouw van zoogdieren en vogels. Zo is de lichaamsoppervlakte-volumeratio hoger bij vogels dan bij zoogdieren, waardoor er bij vogels meer warmteverlies optreedt dan bij zoogdieren. Dit kan resulteren in hypothermie tijdens de ontstekingsreactie, voornamelijk bij Passeriformen. Daarnaast is de gemiddelde lichaamstemperatuur van Passeriformen relatief hoog (41°C), waardoor elke bijkomende temperatuurverhoging door een verhoogd metabolisme moeilijk is, aangezien het metabolisme voor elke stijging van de lichaamstemperatuur met 1°C moet verhogen met 10% (Kluger, 1979; Owen-Ashley en Wingfield, 2007).

Door onderzoek naar de aan- of afwezigheid van veranderingen gerelateerd aan de ontstekingsreactie en naar de omvang ervan, kunnen LPS-inflammatiemodellen toegepast worden in onder andere farmacodynamiekstudies. Hiermee kan het effect van anti-inflammatoire geneesmiddelen, zoals NSAIDs, op de ontstekingsreactie en bijgevolg de klinische relevantie worden nagegaan. Daarnaast kunnen deze modellen ook ingezet worden om de immunomodulerende eigenschappen van andere klassen farmaca te bestuderen, zoals de macrolide antibiotica.

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Diagnostische benadering van cryptorchidie bij de hengst

Diagnostic approach of the cryptorchid stallion

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SAMENVATTING

De diagnose van cryptorchidie bij het paard is vaak een uitdaging aangezien niet altijd definitief uitsluitsel kan gegeven worden op basis van de anamnese, het klinische onderzoek en het rectaal en echografisch onderzoek. Verschillende endocrinologische diagnostische testen zoals de bepaling van het testosteron-, androstenedione-, oestrogenen-, urinaire steroïden- en het antimülleriaans hormoongehalte, die de aanwezigheid van testiculair weefsel aantonen, zijn beschreven. In dit artikel wordt getracht om de voor- en nadelen van deze testen te vergelijken, zodat practici een idee krijgen welke testen in de praktijk gebruikt kunnen worden.

ABSTRACT

The diagnosis of cryptorchidism in horses is often a challenge. Based on the history, clinical and rectal examinations and ultrasonography, a definitive diagnosis is not always possible. Various endocrinological diagnostic assays, such as the determination of testosterone, androstenedione, estrogens, urinary steroids and the anti-Müllerian hormone, which demonstrate the presence of testicular tissue, have been described. These tests all have their advantages and disadvantages, which are discussed in this article in order to help practitioners in the field.

INLEIDING

Bij het veulen dalen de testikels, die zich tijdens de embryonale ontwikkeling caudaal van de nieren bevinden, dertig dagen vóór de geboorte tot twee weken na de geboorte tot in het scrotum (Kristina, 2005). Cryptorchidie of het niet-indalen van één of beide testikels is om verscheidene redenen ongewenst en wordt veelal door middel van castratie verholpen. Door het erfelijke karakter van de aandoening worden dieren met cryptorchidie geweerd uit de fokkerij (Hayes, 1986). Deze afwijking wordt gezien bij 5-8% van de veulens en is meestal unilateraal (Arighi, 2011). Het komt meer voor bij de rassen percheron, saddlebred, quarter horse, bij pony's en bij gekruiste rassen. Bij volbloeden, standardbreds, morgans, Tennessee walking horses en Arabische volbloedrassen daarentegen wordt cryptorchidie minder frequent gezien (Hayes, 1986). Wanneer slechts één testikel is ingedaald (unilaterale cryptorchidie) zijn de paarden meestal vruchtbaar; bilaterale cryptorchen daarentegen zijn steriel (Arighi, 2011). In veertien procent van de gevallen waarbij cryptorchidie vastgesteld wordt, zijn beide testikels niet ingedaald (Hayes, 1986). De niet-ingedaalde testikels kunnen zich intra-abdominaal of inguinaal bevinden. Een laattijdige indaling, op twee- à driejarige leeftijd wordt beschreven maar wordt weinig waarschijnlijk geacht (Cox, 1979). In het geval van inguinale lokalisatie is het meestal de rechtertestikel die niet indaalt, terwijl bij intra-abdominale cryptorchidie het meestal de linkertestikel betreft (Stickle, 1978). Bij pony's is het omgekeerd: de linkertestikel bevindt zich meestal inguinaal en de rechter intra-abdominaal (Kristina, 2005).

De differentiatie tussen een bilateraal gecastreerde hengst of een bilateraal cryptorche hengst (of een unilateraal gecastreerde, unilaterale cryptorch) kan bij paarden met een onbekende voorgeschiedenis of een gebrekkige anamnese een uitdaging vormen.

Waar bij volledig inguinale lokalisatie van de cryptorche testikel de diagnose nog door uitwendige palpatie kan worden gesteld, is bij een intra-abdominale, evenals bij een onvolledig inguinale cryptorch (waarbij de epididymis in het inguinale kanaal ingedaald is terwijl de testikel zich nog intra-abdominaal bevindt), de palpatie moeilijker. Transrectale of transcutane echografie kan worden aangewend, maar gezien de cryptorche testikel veelal hypoplastisch is, kan deze

Tabel 1. De testosterongrenswaarden en beperkingen bij de diagnosestelling van cryptorchidie bij het paard. Testosteronwaarde: nmol/l / 3,47 = ng/ml (Smith et al., 2008).

Hormoon	Staal	Sensitiviteit	Specificiteit	Hengst	Cryptorch	Ruin	Beperkingen
Testosteron	Serum	0,85 [3]	0,91 [3]	>500pg/ml [1] 65-1600pg/ml [2]	<200pg/ml [1] <50-100pg/ml [2]	50-500pg/ml [2]	Meerdere stalen noodzakelijk, + hCG, 14% valspos. of –neg. resultaten[8]

[1]: Mc Cue 2014. De testosteronconcentratie bekomen na hCG-stimulatie bij de hengst, ruin en cryptorch verschilt naargelang de studie. Indien er testiculair weefsel aanwezig is, dan verdubbelt of verviervoudigt de testosteronconcentratie ten opzichte van de basale waarde (i.e. vóór de toediening van hCG). Deze stijging is diagnostisch van belang, niet de waarde zelf. [2]: Cox et al., 1973; [3] Illera et al., 2003 [8] Mueller et al., 1999

diagnostische methode niet steeds uitsluitsel geven. Hengsten zijn doorgaans minder gewend aan rectaal onderzoek, hetgeen het onderzoek meer risicovol kan maken. Daarenboven is bij minirassen de rectale benadering meestal niet mogelijk.

Waar palpatie of echografisch onderzoek tekortschiet, kan veelal definitief uitsluitsel gegeven worden door middel van endocrinologische testen. In dit artikel wordt een overzicht gegeven van de verschillende endocrinologische, diagnostische mogelijkheden, elk met hun specificiteit en sensitiviteit.

ENDOCRINOLOGISCHE TESTEN

Testosteron

Testosteron is een hormoon dat voornamelijk geproduceerd wordt ter hoogte van de leydigcellen van de testes als respons op de pulsatiele secretie van het luteïniserend hormoon (LH) uit de hypofyse (Odell, 1989). Bij de hengst is het testosterongehalte in het bloed individueel verschillend (Pineda, 2003). Het is seizoens- en leeftijdsafhankelijk met de laagste concentraties van september tot januari (Naden et al., 1990). Tijdens de puberteit, rond de leeftijd van 68 weken, vindt een trage stijging van het testosterongehalte plaats en dit tot de leeftijd van 75-80 weken (Naden et al., 1990; Clay et al., 1992). Nadien varieert de concentratie in functie van het seizoen en het moment van de dag.

Het is van belang om het testosterongehalte te bepalen in het bloed. Kleine hoeveelheden testosteron kunnen ook gevonden worden in de urine van ruinen, wat waarschijnlijk het gevolg is van de biosynthese ter hoogte van de adrenale cortex (Silberzahn et al., 1984).

De serum-testosteronconcentratie bij hengsten is meestal hoger dan 500-1000 pg/ml (0,5-1,0 ng/ml) (Mc Cue, 2014), hoewel minima van 65 en maxima van 1600 pg/ml ook worden vermeld (Cox et al., 1973). Bij een ruin daarentegen is de concentratie lager dan 50-100 pg/ml (0,05-0,10ng/ml), terwijl de testosteronconcentratie bij een cryptorche hengst, tussen beide waarden ligt (50-500 pg/ml), maar veelal minder dan 200 pg/ml bedraagt (Mc Cue, 2014). Soms worden overlappende waarden waargenomen zoals lage testosteronconcentraties bij hengsten en hoge bij ruinen (Cox et al., 1973). In twee studies van Arighi et al. (1985; 1989) werd geconcludeerd dat een waarde van <0,24 ng/ml diagnostisch is voor de afwezigheid van testiculair weefsel, terwijl concentraties van >0,44 ng/ml indicatief zijn voor de aanwezigheid van testiculair weefsel (Arighi et al., 1985; Arighi et al., 1989). Bij waarden tussen 0,24 ng/ml en 0,44 ng/ml wordt een andere diagnostische methode aangeraden (Tabel 1).

Gonadotropine releasing hormoon (GnRH), en dus ook LH, worden in pulsen vrijgesteld, waardoor het testosterongehalte in de loop van de dag schommelt (Nett, 1993). In de ochtend is de concentratie het laagst, waarna ze toeneemt en rond de middag het hoogst is (McCue, 2014). Een eenmalige testosteronbepaling is dus weinig informatief. Indien er maar één bloedstaal genomen wordt, wordt dit het beste gedaan in de vroege namiddag, op het moment dat de circulerende hormonen het hoogst zijn (McCue, 2014).

Door de diurnale en seizoensvariatie is een bepaling van de testosteronconcentratie na een hCG-stimulatietest meer aangewezen (Cox et al., 1975). In tegenstelling tot ruinen, waarbij geen respons waarneembaar is, vertonen hengsten en cryptorchen een graduele stijging van de testosteronconcentratie in het serum na hCG-stimulatie (Silberzahn et al., 1989). Deze stijging is het gevolg van een verhoogde secretie van testosteron door de leydigcellen na hCG-stimulatie (Amann, 1981). Wat van belang is bij de hCG-stimulatietest is de stijging van de concentratie ten opzichte van de basale waarde. Deze stijging is variabel en kan na 0 tot 120 minuten verdubbelen tot verviervoudigen (McCue, 2014). De locatie van de testis, de leeftijd van het dier, evenals het moment van staalname (dagritme, seizoen) beïnvloeden de respons na hCG-toediening (Cox et al., 1975; Arighi et al., 1985; Arighi et al., 1989).

De hCG-stimulatietest kan op twee verschillende manieren uitgevoerd worden (McCue, 2014). De eerste manier betreft een eenmalige bloedafname vóór een injectie van hCG, om de basale testosteronwaarde te bepalen, waarna 6-12 000IU hCG IV worden toegediend. Eén en twee uur na de behandeling wordt telkens een bloedstaal genomen. Gezien de soms laat-

Tabel 2. Androstenedionegrenswaarden en -beperkingen bij de diagnosestelling van cryptorchidie bij het paard.

Hormoon	Staal	Sensitiviteit	Specificiteit	Hengst	Cryptorch	Ruin	Beperkingen
Androstenedione	Serum	0,92 [3]	0,93 [3]	10,52 ng/ml [3] 0,55-0,70 ng/ml [2]	0,51 ng/ml [3] 0,180-0,350 ng/ml [2]	0,03 ng/ml [3] 0,079-0,081 ng/ml [2]	niet routinematig aangeboden

Cox et al., 1973; [3] Illera et al., 2003

tijdige reactie bij abdominaal cryptorche dieren wordt er 24 uur na de hCG-toediening een derde staal genomen. De diagnose van testiculair weefsel hangt af van de stijging in plasmatestosteron na hCG-stimulatie.

Een tweede protocol behelst een bloedafname dertig minuten vóór de toediening van 10 000IU hCG evenals op het moment van de toediening. Vervolgens wordt er om de dertig minuten bloed afgenomen en dit tot drie uur na de toediening.

Echter, Illera et al. (2003) toonden aan dat bij de bepaling van testosteron na stimulatie een hoog aantal (15%) valsnegatieve resultaten gevonden wordt, met een lage, negatief voorspellende waarde (0,85) en een lage sensitiviteit (0,85) in vergelijking met oestronsulfaat- en androstenedione-detectie (Illera et al., 2003) voor wat betreft de aanwezigheid van testiculair weefsel. Ook in een studie van Cox et al. (1986) konden de resultaten in 6,7% van de gevallen niet eenduidig geïnterpreteerd worden.

ANDROSTEENDION

Androsteendion is, zowel bij mannelijke (in de testes) als vrouwelijke (in de ovaria) dieren, een precursorhormoon van androgenen en oestrogenen. Het is betrokken bij de regulatie van onder andere de puberteit, de controle van de folliculaire groei en de mannelijke, reproductieve fysiologie (Inoue et al., 1993; Muyan et al., 1993; Hoffman en Landeck, 1999). De androsteendionconcentratie in het serum kan bepaald worden aan de hand van een "amplified enzyme immunoassay" (Illera et al., 2003). De concentratie is het hoogst bij de hengst en het laagst bij de ruin. Cryptorchen hebben een concentratie tussen die van hengst en ruin (Tabel 2). Er werd aangetoond dat de bepaling van androsteendion samengaat met een hogere sensitiviteit (0,92) en specificiteit (0,93), en dat de test efficiënter (0,92) is dan de bepaling van testosteron en oestronsulfaat. De meeste commerciële labo's bieden deze analyse echter niet aan (Tabel 2).

URINAIRE STEROÏDEN

De diagnose van cryptorchidie kan ook gesteld worden wanneer de steroïden 5(10)-estrene-3β,17α-diol en/of 4-estren-3,17-dion in de urine van vermeende ruinen aanwezig zijn. 5(10)-Estren-3β,17α-diol kan niet worden aangetoond in de urine van de ruin en 4-estren-3,17-dion is in een grotere concentratie aanwezig bij hengsten en cryptorchen. Deze twee steroïden worden geproduceerd tijdens de biosynthese van androgenen en worden gedetecteerd aan de hand van gaschromatografie en een massaspectrometrietest (Leung et al., 2011). Deze test is in tegenstelling tot de bepaling van estronsulfaat toepasbaar bij paarden vanaf twee jaar. Bovendien kunnen ook andere testiculaire, steroïdale merkers aangetoond worden, onder andere testosteron, nandrolon, estron en 17β-estradiol. Deze vier zijn echter minder specifiek maar indien ze in grote hoeveelheden aanwezig zijn in de urine, kunnen ze gebruikt worden als bijkomend bewijs voor de aanwezigheid van testiculair weefsel (Leung et al., 2011). Dergelijke analyses worden echter niet standaard aangeboden in hematologische routinelaboratoria.

OESTROGENEN

Oestrogenen worden na aromatisatie gevormd uit testosteron. Dit vindt bij de hengst, in tegenstelling tot bij andere diersoorten, plaats ter hoogte van het testiculaire weefsel (Pineda, 2003). Of dit aromatisatieproces plaatsvindt ter hoogte van de sertoli- en/of de leydigcellen is nog een punt van discussie (Hejmej et al., 2005). De gevormde oestrogenen kunnen onderverdeeld worden in gebonden oestrogenen na conjugatie, d.i. oestronsulfaat, en ongebonden oestrogenen of oestradiol 17 β (Schonert et al., 2012). Achtennegentig procent van de geproduceerde oestrogenen is gebonden oestrogeen (Raeside, 1978). Oestradiol 17 β heeft invloed op meerdere orgaansystemen en zou verantwoordelijk zijn voor het hengstengedrag (Schonert et al., 2012). Zoals dit het geval is voor testosteron is ook de concentratie van oestrogenen in het bloed verschillend tijdens het jaar. De concentratie is het hoogst in mei en het laagst in november en december (Raeside, 1978), wat samenhangt met het voortplantingsseizoen van paarden. De intratesticulaire hoeveelheid oestradiol stijgt met de testiculaire maturatie (Stewart en Roser, 1998).

De bepaling van de hoeveelheid oestrogenen kan ook waardevol zijn bij het diagnosticeren van een cryptorch. Aangezien 98% van de geproduceerde oestrogenen gebonden is (Raeside, 1978), is het bepalen van ongebonden oestrogenen niet relevant. De bepaling van plasma-oestronsulfaat aan de hand van een radio-immuno-assay (RIA) wordt aangewend als diagnostisch middel voor het aantonen van testiculair weefsel. Wanneer de gemeten concentraties hoger

zijn dan 400 pg/ml, is het paard zeker cryptorch (Silberzahn et al., 1989). Indien de waarde lager is dan 50 pg/ml, is het paard zeker een ruin. Voor waarden tussen dit interval is een andere diagnostische test aangewezen. Deze test is niet toepasbaar bij paarden jonger dan drie jaar aangezien de testiculaire productie van oestronsulfaat verwaarloosbaar is bij jonge hengsten (Gaillard en Silberzahn, 1987). Afhankelijk van de gebruikte antilichamen in de verschillende testen worden echter verschillende drempelwaarden waargenomen. In een studie van Arighi en Bosu (1989) was de oestronsulfaatconcentratie <0,12 ng/ml (<0,34 nmol/l) bij ruinen en >1ng/ml (>2,85 nmol/l) bij dieren met testiculair weefsel. Palme et al. (1994) daartegen beschreven bij deze dieren hogere concentraties (respectievelijk <3,5 nmol/l en >37 nmol/l) (Tabel 3). Een voorafgaande injectie met hCG heeft weinig zin daar hCG bijna geen kortetermijneffect vertoont op oestrogenen van hengsten en cryptorchen (Ganjam en Kenney, 1975). Unilaterale cryptorchen hebben een hogere ($p<0,05$) concentratie geconjugeerde oestrogenen in het bloed dan bilateraal of unilateraal gecastreerde paarden (Palme, 1994). Bij de ezel kan de diagnose van cryptorchidie aan de hand van oestronsulfaat niet gebruikt worden, aangezien deze geen geconjugeerde oestrogenen hebben (Cox et al., 1986).

In een studie van Illera et al. (2003) werd de concentratie van oestronsulfaat in het serum vergeleken met deze van testosteron en androsteendion. Uit deze vergelijking bleek dat de positief voorspellende waarde (0,85) voor oestronsulfaat lager was, de hoeveelheid valspositieve resultaten hoger en de specificiteit lager dan voor beide andere hormonen, wat betreft de aanwezigheid van testiculair weefsel.

De bepaling van fecale ongeconjugeerde oestrogenen kan ook gebruikt worden als diagnostisch middel. In een studie van Palme et al. (1994) werden hengsten, ruinen en cryptorchen onderzocht. De hoeveelheid fecale oestrogenen bij de hengsten was gemiddeld 130 nmol/kg, terwijl dit bij de ruinen gemiddeld 12 nmol/kg was. De hengsten met één niet-ingedaalde testikel hadden een gemiddelde concentratie van 113 nmol/kg en de hengsten waarbij beide testikels niet ingedaald waren 64 nmol/kg (Tabel 3). Paarden met minstens één scrotale testikel hebben dus significant hogere ($p<0,05$), fecale oestrogeenconcentraties dan cryptorchen zonder scrotale testes (Palme et al.,1994). Dit wijst erop dat niet-ingedaalde testes minder oestrogenen produceren (Cox et al., 1986). Het oestrogenengehalte van deze paarden met minstens één scrotale testikel is nagenoeg gelijk aan deze bij hengsten, waardoor de aan- of afwezigheid van een cryptorche testes bij paarden met een normale intrascrotale testikel niet gediagnosticeerd kan worden (Palme, 1994). Ook deze test is niet bruikbaar bij paarden jonger dan twee jaar (Palme et al., 1994). Aangezien de oestrogeenconcentratie in feces varieert gedurende de dag, wordt aangeraden meer dan één fecesstaal te analyseren voor de bepaling van de oestrogeenconcentratie (Palme et al., 1994). Fecale oestrogenen zijn gedurende minstens één week stabiel en kunnen dus opgestuurd worden naar het labo waar ze aan de hand van een radio-immuno-assay onderzocht worden (Choi, 1987). De accuraatheid van de bepaling van fecale oestrogenen komt overeen met de accuraatheid van de bepaling van de concentratie van testosteron (na hCG-stimulatie) en de bepaling van oestronsulfaat in het bloed (Palme et al., 1994).

Naast de bepaling van oestrogenen in bloed en feces kan ook de hoeveelheid oestrogenen in de urine bepaald worden om de aanwezigheid van testiculair weefsel te bevestigen. De hoeveelheid oestrogenen in de urine stijgt van november tot mei (Palme et al., 1998). Bij ruinen is de gemiddelde oestrogenenconcentratie in de urine 11,3 nmol/l. Zowel bij hengsten als bij cryptorchen is de gemiddelde concentratie 2000 tot 10 000 maal hoger (respectievelijk 14 500 nmol/l en 66 000 nmol/l) (Palme et al., 1998) (Tabel 3).

SERUM-ANTIMÜLLERIAANS HORMOON

Het antimülleriaans hormoon (AMH) is een glycoproteïne gelinkt aan een disulfide dat behoort tot de "transforming growth factor beta"-familie. Bij mannelijke dieren wordt het in het vroege foetale leven gesecreteerd door de sertolicellen. In de prepuberale testes is de hoeveelheid lager (Ball et al., 2008; Almeida et al., 2012).

De normale sertolicelmaturatie in de testis gaat gepaard met een gedaalde AMH-expressie (Almeida et al., 2012). In de niet-ingedaalde testis vindt de matu-

Tabel 3. Oestrogeengrenswaarden en -beperkingen bij de diagnosestelling van cryptorchidie bij het paard.

Hormoon	Staal	Sensitiviteit	Specificiteit	Hengst	Cryptorch	Ruin	Beperkingen
Oestrogenen	Serum (Oestone)	0,88 [3]	0,84 [3]	175 ng/ml [3] 45-60 ng/ml [4]	58,53 ng/ml [3] 0,30-0,55 ng/ml [4]	0,07 ng/ml [3] 0,025-0,035 ng/ml [4]	Niet toepasbaar bij paarden < 3 jaar
	Urine	-	-	145000 nmol/l [7]	66000 nmol/l [7]	11,3 nmol/l [7]	
	Feces	-	-	130 nmol/kg [5]	Unilat.: 113 nmol/kg [5] bilat.: 64 nmol/kg [5]	12 nmol/kg [5]	Niet toepasbaar bij paarden < 2 jaar

[3]: Illera et al., 2003; [4]: Silberzahn et al., 1989; [5]: Palme, 1994; [7]: Palme et al., 1998

Tabel 4. Grenswaarden en beperkingen van het antimülleriaans hormoon bij de diagnosestelling van cryptorchidie bij het paard.

Hormoon	Staal	Hengst	Cryptorch	Ruin	Beperkingen
AMH	Serum	15 ng/ml [6]	32,5 ng/ml [6]	< detectielimiet	niet toepasbaar bij prepuberale dieren[9]

[6]: Claes et al., 2013; [9]Ball et al., 2008

ratie van sertolicellen onvoldoende plaats (Almeida et al., 2012), waardoor de hoeveelheid AMH in het serum, bepaald aan de hand van een "enzyme linked immunosorbent assay" (ELISA), bij een cryptorch hoger is dan bij een hengst (Claes et al., 2013). De AMH-concentratie bij ruinen ligt onder de detectielimiet. Er dient opgemerkt te worden dat de hoeveelheid AMH bij een cryptorch dezelfde is als deze bij hengstenveulens vóór de puberteit (Ball et al., 2008; Almeida et al., 2012). Naast leeftijd heeft ook het seizoen een invloed op de AMH-concentratie, met een piek in mei en een dieptepunt in november (Claes et al., 2013).

Gemiddeld is het gehalte aan AMH in het bloed bij intacte hengsten 13,3 ± 1,8 ng/ml (range 1,7 ng/ml en 21,9 ng/ml) (Murase, 2015), met hogere waarden in mei en juni (Claes et al., 2013). Deze stijging komt overeen met de stijging van de gonadotropinen en de steroïd hormonen (Taya et al., 2000). De hoeveelheid AMH bij een unilaterale (hemi-gecastreerde) hengst is 17,6 ± 3,0 ng/ml (range 3,1 ng/ml en 28,2 ng/ml) (Murase, 2015). Claes et al. (2013) rapporteerde gelijkaardige waarden van 15 ng/ml bij de hengst en 32,5 ng/ml bij een cryptorch (Tabel 4). Deze kleine verschillen kunnen te wijten zijn aan verschillen in leeftijd en de aan- of afwezigheid van een tweede testikel (Murase, 2015). In sommige gevallen wordt aangetoond dat cryptorche dieren met een testosteronconcentratie die duidt op de afwezigheid van testiculair weefsel, toch een positieve AMH-test vertoonden. De bepaling van AMH is dus een belangrijk middel in het geval van twijfelachtige gevallen (Claes et al., 2014). De AMH-concentratie is hoger bij bilaterale cryptorchen dan bij unilaterale cryptorchen.

CONCLUSIE

Iedere diagnostische methode heeft zijn waarde. De drie meest bruikbare endocrinologische methoden in de praktijk zijn de bepaling van de basale testosteronconcentratie, de hCG-stimulatietest en de bepaling van AMH. De bepaling van geconjugeerde oestrogenen is 96% accuraat voor de bepaling van cryptorchidisme (Arighi et al., 1989) maar niet bruikbaar bij jonge dieren. Wanneer men een cryptorch wenst te onderscheiden van een ruin aan de hand van de bepaling van de basale testosteronconcentratie, wordt er in 14% van de gevallen een verkeerde diagnose gesteld (Mueller et al., 1999). De bepaling van de testosteronconcentratie na de hCG-stimulatietest is 94,6% accuraat voor het aantonen van testiculair weefsel (Cox, 1975). In tegenstelling tot de productie van testosteron vindt de productie van AMH alleen plaats ter hoogte van de sertolicellen. De bepaling van AMH is dus specifieker voor de diagnose van testiculair weefsel (Claes et al., 2014). Een bijkomend voordeel is dat er maar één staal nodig is voor de bepaling van AMH, dit in tegenstelling tot de bepaling van testosteron na hCG-stimulatie, waarbij meerdere stalen nodig zijn (Claes et al., 2014). Er dient opgemerkt te worden dat er bij de interpretatie van hormonale resultaten rekening moet gehouden worden met de referentiewaarden en de verschillende detectiemethoden die gebruikt worden door de verschillende laboratoria (Mueller et al., 1999).

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BOTERPATER ONTTOVERT HOFSTEDE

We zijn thuis een keer betovert geweest en dat heeft een hele tijd geduurd (...). Wij bouwden een nieuwe stal, helegans modern, groot, met alle mogelijke moderne dingen. Wij kochten nieuwe beesten, koeien, paarden, varkens, konijnen kiekeren (kippen), allee dat we een serieuze boerderij hadden. Nu, als de stal daar stond, komt er een vrouw op het hof. 'Ah,' zegt ze 'ik heb gehoord st ge zulk een schone stal hebt, mag ik ne keer komen zien?' Maar omdat ze de naam had van toveres, werd ze niet in de stal toegelaten.

Maar van dan af kregen we het ene malheur na het andere. Dat hield niet op. de beesten werden ziek, het ene na het andere. Ze stierven aan de pokken. Onze koeien miskalfden of bleven met de berre (nageboorte) *op staan en stierven. Als ons paard moest een veulen afroeien* (veulenen), *bleef het ook met de berre op staan en stierf nadatum ook. Twee zeugen misviggerden na elkander en stierven. Ook onze konijnen kregen de pokken en stierven. (...). Ge moet niet vragen dat ons dat veel geld kostte.*

Nu elk jaar kwam de boterpater rond en hij kwam ook bij ons elk jaar eten en iets drinken en een sigaar opsteken. Toen hij daar kwam, vertelde mijn moeder alles nadat hij gegeten had. 'Madammeke,' zei hij, 'dat zal wel allemaal goed komen. 'Ge moet een beetje courage hebben.' 'Ja maar meneer de pater, zei mijn moeder, 'ze zijn ons grat (totaal) *aan het renueren* (ruïneren).'

Maar dat beterde niet. Al die malheuren duurden voort. 't Jaar daarachter, toen de boterpater weer kwam, zegde mijn moeder dat weer hé, dat ze nu nagenoeg helegans plat gerenueerd waren op de boerderij. En de pater ging na het eten heel ons huis en het hof af. Hij begon op de zolder eerst, hij had de duivelbezwering van de heilige Antonius in zijn hand. dat was zo een blikken kruiske. Hij hield dat in zijn hand onder zijn mouw en zo ging hij rond. Alle slaapkamers overlas hij, de winkel, tot in de kelder toe, alle kamers van ons huis, geheel de hofstede. Het laatst kwam hij aan de remise. Daar was een oven in de hoek. En daar bleef hij heek lang staan, wel twintig minuten. Toen ging hij achterwaarts het hof af. En hij zei dat het nu wel zou beteren. Mijn moeder vroeg of hij nog iets wilde drinken, maar hij zei dat het nu niet meer kon. 'Allee', zegt mijn vader, 'nu wil ik toch eens zien hoe dat het zit.' En wij hadden nog een koe staan. Wij gingen die laten onderzoeken en die was zo zuiver als iets. Daar mankeerde niets meer aan. En zo is dat helemaal gebeterd.

Bron:

Top, S., (2006). *Op Verhaal komen. Oost-Vlaams sagenboek*, Davidsfonds, Leuven, p. 161-162.

Gebaseerd op een verhaal verteld door een werkman uit Moorsel. Opgenomen in de scriptie (Leuven) van Pauwels, L., (1969)), *Sagenonderzoek ten oosten van Aalst en in Noord – West – Brabant* (NvdR: 'boterpaters' waren broeders of paters van bedelorden, meest franciscanen, die jaarlijks op bedeltocht gingen om boter, eieren en andere aalmoezen te verkrijgen op de boerderijen in de gemeenten rond hun klooster).

Luc Devriese

Chirurgische behandeling van een sublinguale sialocoele (ranula) bij een kat

Surgical treatment of a sublingual sialocele (ranula) in a cat

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SAMENVATTING

In deze casuïstiek wordt een zeven jaar oude, mannelijke, gecastreerde, Europese korthaar met een ranula beschreven. De patiënt werd aangeboden vanwege een sublinguale zwelling aan de rechterkant die chirurgisch werd behandeld door middel van marsupialisatie en excisie van de mandibulaire en sublinguale speekselklieren. Histopathologisch onderzoek van de verwijderde weefsels bevestigde de diagnose van een ranula en toonde de onopzettelijke resectie van de rechter submandibulaire lymfeknopen. Vijf maanden na de ingreep werden er geen complicaties of recidieven vastgesteld.

ABSTRACT

In this case report, a seven-year-old, male, castrated European Shorthair diagnosed with a ranula is described. The patient was presented with a sublingual swelling on the right side, which was surgically treated with marsupialization and removal of the mandibular and sublingual salivary glands. Histopathological examination confirmed the diagnosis of a ranula and revealed the accidental resection of the right submandibualr lymph nodes. Five months after the operation, no complications or recurrences were observed.

INLEIDING

Sialocoeles of speekselmucocoeles ontstaan door lekkage van speeksel uit een beschadigde speekselklier of afvoergang (Smith, 2000; Radlinsky, 2013; Langley-Hobbs, 2014). De opstapeling van speeksel in bindweefsel lokt een inflammatoire reactie uit met de vorming van granulatieweefsel tot gevolg (Smith, 2000; Radlinsky, 2013; Langley-Hobbs, 2014). Een sublinguale sialocoele wordt ook een ranula genoemd. Daarnaast zijn er cervicale, faryngeale en zygomatische sialocoeles.

Uit gerapporteerde gegevens blijkt dat de gemiddelde incidentie van speekselklieraandoeningen bij honden en katten ongeveer 0,3% is, waarbij honden vaker zijn aangetast dan katten (Brown, 1989; Spangler en Culbertson, 1991; Waldron en Smith, 1991; Hammer et al., 2001). Sialocoeles zijn de meest voorkomende afwijkingen van de speekselklieren.

Klinische symptomen van sialocoeles bij de hond of kat zijn afhankelijk van de locatie van de sialocoele. Sublinguale sialocoeles geven een fluctuerende, niet-pijnlijke zwelling onder de tong. Er kunnen moeilijkheden bij eten, mondbloedingen, intermitterend braken, anorexie, ptyalisme en/of lethargie optreden (Radlinsky, 2013). Als een sublinguale of faryngeale sialocoele zeer groot is, kan deze ook aanleiding geven tot respiratoire stridor (Kiefer en Davis, 2007; Dietens et al., 2011).

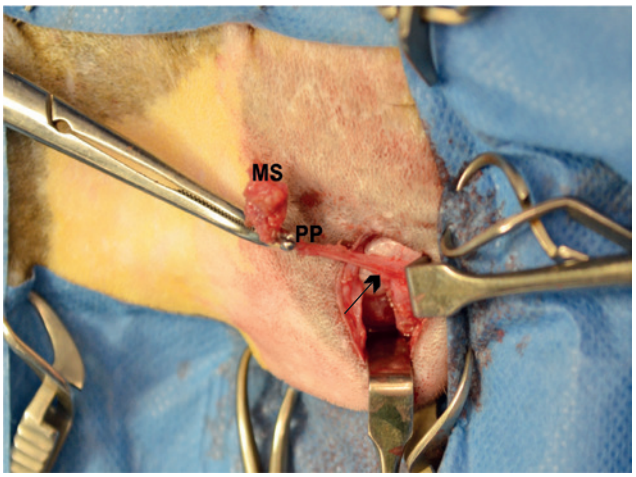
Ter behandeling van een sublinguale sialocoele zijn er vier opties en/of combinaties mogelijk: drainage (Kilic, 2009), injectie met cauteriserende/anti-inflammatoire producten (Saifzadeh, 2004; Radlinsky, 2013), marsupialisatie (Lane, 2012) en sialoadenectomie (Radlinsky, 2013).

In deze casus wordt een volwassen kater met een ranula beschreven die succesvol behandeld werd met excisie van de speekselklier in combinatie met marsupialisatie. In de discussie wordt de regionale anatomie bij de kat besproken en wordt verder de nadruk gelegd op de diagnosestelling en chirurgische behandelingsopties van deze aandoening.

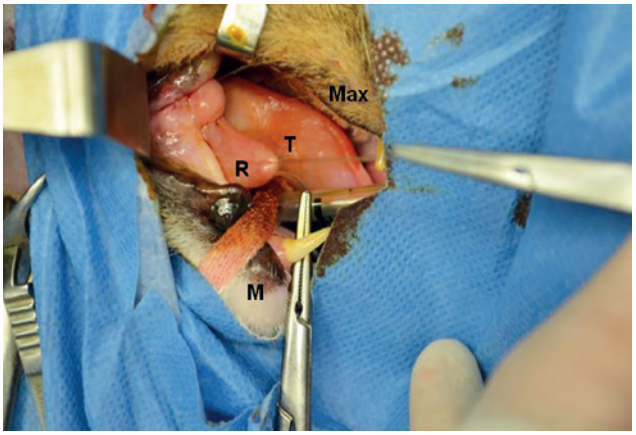
CASUÏSTIEK

Een zeven jaar oude, mannelijke, gecastreerde Europese korthaar van 6,6 kg werd aangeboden op de

Figuur 1. Een zeven jaar oude, mannelijke, gecastreerde Europese korthaar werd aangeboden met een sublinguale zwelling aan de rechterkant.



Figuur 2. Caudale tractie op de mandibulaire speekselklier (MS) met behulp van een weefselklem, zodat de afvoergang met aanpalende, sublinguale speekselklier (pars polystomatica, PP) gedisseceerd kon worden. De afvoergang (pijl) loopt verder in de diepte. De handretractor trekt de spierbuik van de musculus digastricus naar ventraal en zorgt zo voor een betere visualisatie. Rostraal is rechts.



Figuur 3. Rechts lateraal aanzicht van open mond met steunhechting in het dak van de ranula (R). Onderzijde tong (T), maxilla (Max) en mandibula (M) worden weergegeven op de foto als oriëntatiepunten.

Faculteit Diergeneeskunde in Merelbeke (UGent) ter behandeling van een sublinguale intraorale zwelling rechts.

Reeds een week at de kat moeilijker waarbij hij zijn kop telkens naar links hield, drinken ging zonder problemen. De eigen dierenarts stelde een zwelling vast rechts onder de tong en stuurde de kat door na het opstarten van meloxicam 0,05 mg/kg (Loxicom®, Norbrook lab, Newry, Noord-Ierland) en pradofloxacine 5 mg/kg (Veraflox®, Bayer, Leverkusen, Duitsland), beide orale suspensies. De ingestelde behandeling, die één week werd aangehouden, had geen effect op de grootte van de zwelling noch op het eetgedrag.

De kat was alert en een fluctuerende, niet-pijnlijke zwelling rechts onder de tong werd waargenomen (Figuur 1). Verder waren er geen afwijkingen, uitgezonderd een te hoge lichaamsconditiescore van 8 op 9.

Op basis van de anamnese en het lichamelijk onderzoek werd de waarschijnlijkheidsdiagnose van een ranula gesteld. Er werd geadviseerd om een marsupialisatie van de ranula en sialoadenectomie van de mandibulaire en sublinguale speekselklieren uit te voeren.

Een maand na het stellen van de diagnose werd de patiënt aangeboden voor de chirurgische correctie van de ranula. De kat werd intraveneus (IV) gepremediceerd met methadon 0,1 mg/kg (Comfortan®, Eurovet A.H., Bladel, Nederland) en dexmedetomidine 2 µg/kg (Dexdomitor®, Orion Corporation, Espoo, Finland). Vervolgens werd hij geïnduceerd met propofol 3 mg/kg IV (Propovet Multidose®, Abbott Lab, Berkshire, Verenigd Koninkrijk). Het onderhoud van de anesthesie gebeurde met isofluraan 1,3 – 1,5% (Isoflo®, Abbott Lab, Berkshire, Verenigd Koninkrijk) verdampt in 100% O_2. Gedurende de operatie werd bijkomende analgesie bekomen door middel van "constant rate infusion" (CRI) van fentanyl 5 – 7 µg/kg/h (Fentadon®, Eurovet, Bladel, Nederland). Profylactisch werd cefazoline 20 mg/kg IV (Cefazoline®, Sandoz, Vilvoorde, België) gegeven.

De rechter hals- en kopregio werden ruim geschoren. De patiënt werd in links laterale decubitus gepositioneerd met een opgerolde handdoek onder de nek. De operatieplaatsen, namelijk kaakregio en ranula, werden aseptisch voorbereid met povidone-jood-zeepoplossing (Vetclean®, Ecuphar, Oostkamp, België) en pure joodoplossing (Povidone dermicum®, Ecuphar, Oostkamp, België). De mandibulaire speekselklier werd gepalpeerd achter de kaaktak ter hoogte van de samenvloeiing van de rechter vena linguofacialis en vena maxillaris in de rechter vena jugularis externa. Op deze plaats werden de huid en subcutis van dorsaal naar ventraal ingesneden. Hemostase werd bekomen door middel van bipolaire elektrocoagulatie. Vervolgens werd de musculus platysma ingesneden en werd er stomp vrijgeprepareerd tot op het kapsel van de mandibulaire speekselklier. Het kapsel werd ingesneden en de speekselklier werd losgemaakt van het kapsel, waarbij mediaal gelegen bloedvaten werden gecoaguleerd. Door middel van caudale tractie op de mandibulaire speekselklier verliep de dissectie van

de sublinguale speekselklier vlotter (Figuur 2). De afvoergang werd verder naar craniaal gevolgd langs de musculus digastricus tot de linguale zenuw geïdentificeerd kon worden. Ligatie en transectie van de ductus gebeurden net caudaal van de nervus lingualis. De sluiting van de wonde gebeurde in drie lagen. Eerst werd een doorlopende hechting uit polyglecaprone 25 4/0 (Monocryl®, Ethicon, Diegem, België) geplaatst op de musculus platysma. Daarna werden met hetzelfde hechtmateriaal ook de subcutis (matrashechting) en de huid (intradermale hechting) gesloten.

Vervolgens werd overgegaan tot marsupialisatie van de ranula. Eerst werd een steunhechting geplaatst op het dak van de ranula (Figuur 3). Daarna werd een incisie gemaakt door de volledige dikte van de wand (Figuur 4). Het dak van de ranula werd verwijderd en de sectieranden van het granulatieweefselkapsel werden vastgehecht aan de mondmucosa door middel van twee doorlopende hechtingen van rostraal naar caudaal met polyglecaprone 25 5/0. Alle verwijderde weefsels werden opgestuurd voor histopathologisch onderzoek.

Na de operatie werd de kat gehospitaliseerd. Postoperatieve analgesie werd bekomen met buprenorfine 0,02 mg/kg IV qid (Vetergesic®, Alstoe, York, Verenigd Koninkrijk) en meloxicam 0,2 mg/kg subcutaan (SC) sid (Metacam®, Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Duitsland). De antibioticumkuur met cefazoline 20 mg/kg tid werd aanvankelijk intraveneus verdergezet. Een dag na de operatie begon de kat te eten; hierna werd beslist dat hij de volgende dag naar huis mocht. Er werd aangeraden om thuis gedurende tien dagen zachte voeding aan te bieden (blikvoeding of geweekte korrels). De antibioticumbehandeling werd nog zeven dagen verdergezet met cefalexine 15 mg/kg per os bid (Therios®, Sogeval Lab, Laval, Frankrijk) en meloxicam 0,05 mg/kg per os sid werd nog gedurende drie dagen toegediend om de pijn en ontsteking te onderdrukken.

Uit het histopathologisch onderzoek bleek dat naast de speekselklieren, bijhorende afvoergangen en het ranuladak, ook de submandibulaire lymfeknopen verwijderd waren. De speekselklieren en hun afvoergang vertoonden op het histologisch onderzoek een normaal aspect. In het weefselfragment van de mondmucosa, dat het dak van de ranula uitmaakte, waren uitmondingen van afvoergangen te zien. Ook werden fistelgangen met een uitgesproken pyogranulomateuze ontsteking opgemerkt. De lymfeknopen vertoonden uitgesproken lymfoïde hyperplasie met vorming van duidelijke kiemcentra en bevatten nesten van mastcellen die suggestief waren voor mastocytose of metastasen van een regionale primaire mastceltumor.

Twee weken na de operatie werd de patiënt ter controle aangeboden. De eigenaars meldden dat de kat thuis alert was en het eten en drinken vlot verliepen. Ongeveer een week na de operatie werd er eenmalig een beetje bloed gezien in de muil. Tijdens het algemeen lichamelijk onderzoek werden geen afwijkingen gevonden. Het operatielitteken ter hoogte van de huid was droog en gesloten. Ter hoogte van de kop of de nek werd geen oorzaak gevonden van de aanwezigheid van de mastcellen in de mandibulaire lymfeknopen.

Figuur 4. Rechts lateraal aanzicht van open mond met een kompres ter absorptie van bloed en een steunhechting in de ranula. De volledige dikte van de sialocoelewand werd ingesneden.

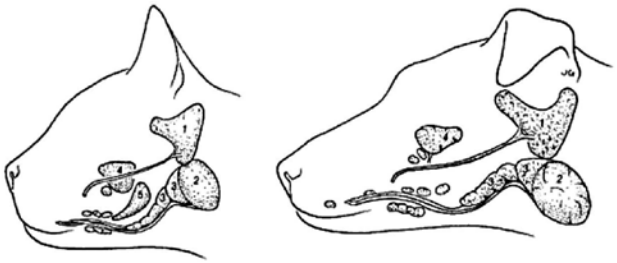
De kat kwam vijf maanden postoperatief een laatste keer op controle. Er waren geen klinische klachten of recidieven van de ranula.

DISCUSSIE

Sublinguale sialocoeles ontstaan meestal ten gevolge van beschadiging van de sublinguale speekselklier of de afvoergang ervan, waarbij speeksel geaccumuleerd wordt in de mondbodem (Smith, 2000; Lane, 2012; Radlinsky, 2013; Langley-Hobbs, 2014). Doordat het vrijgekomen speeksel mild irriterend is vanwege de enzymen die er zich in bevinden, ontstaat er een ontstekingsreactie (Lane, 2012). In tweede instantie wordt granulatieweefsel gevormd. Het speeksel wordt op deze manier omkapseld zodat verdere migratie voorkomen wordt (Lane, 2012; Radlinsky, 2013).

Etiologieën van ranulae bij katten zijn nog niet volledig opgehelderd (Kiefer en Davis, 2007; Lane, 2012). Bovendien worden ze ook zelden geïdentificeerd. Hoewel trauma als belangrijkste oorzaak vooropgesteld wordt bij honden en katten, zijn er hiervoor meestal geen bewijzen (Lane, 2012; Radlinsky, 2013). Andere oorzaken die gesuggereerd worden zijn vreemde voorwerpen, stenose of obstructie van de speekselklierafvoergang en sialolieten (Bellenger en Simpson, 1992; Vallefuoco et al., 2011; Radlinsky, 2013). In deze casus was er een vermoeden van trauma, maar de exacte etiologie werd ook hier niet achterhaald.

De anatomie van de speekselklieren van katten is vrij beperkt en weinig gedetailleerd beschreven in de huidige literatuur. De anatomie van kop en speek-



Figuur 5. Schematische voorstelling van de belangrijkste speekselklieren bij de kat (links) en de hond (rechts). 1. Parotis speekselklier; 2. Mandibulaire speekselklier; 3. Sublinguale speekselklier, pars monostomatica; 4. Zygomatische speekselklier; 5. Molaire speekselklier. De niet-geïdentificeerde speekselklier gelegen rondom afvoergangen van sublinguale en mandibulair klieren is de sublinguale speekselklier, pars polystomatica (uit: Dunning, 2003).

selklieren van de hond is daarentegen vakkundig onderzocht en uitgebreid beschreven. De algemene anatomische kenmerken zijn wel vermeld bij katten. Katten bezitten vijf paar speekselklieren: de glandulae parotis, mandibularis, sublingualis, zygomaticus en molaris (Figuur 5). Deze laatste is uniek voor de kat en komt niet voor bij de hond en evenmin bij de mens (Dunning, 2003; Kiefer en Davis, 2007; Langley-Hobbs, 2014). De parotisspeekselklier (ventraal van het oorkanaal), de glandula zygomaticus (tussen de jukboog en oogkas) en de glandula molaris (tussen lipmucosa en m. orbicularis oris) met bijhorende afvoergangen kunnen weliswaar aanleiding geven tot een mucocoele maar zijn van geen belang bij het ontstaan van ranulae. De sublinguale speekselklier met bijhorende afvoergangen daarentegen liggen meestal aan de basis hiervan (Smith, 2000; Lane, 2012; Langley-Hobbs, 2014). De sublinguale speekselklier bij de hond en kat bestaat uit een pars monostomatica en een pars polystomatica. De lobuli van de pars monostomatica van de hond en kat hebben zich rond de mandibulaire en sublinguale afvoergang ter hoogte van de tongwortel georganiseerd. De sublinguale speekselklier pars monostomatica bestaat bij de hond uit een caudaal en rostraal deel. Het caudale deel is verbonden en ligt in een gemeenschappelijk bindweefselkapsel met de mandibulaire speekselklier (Peeters, 1991). Beide delen van de pars monostomatica van de sublinguale speekselklier draineren in één sublinguale afvoergang (Peeters, 1991; Dunning, 2003). De lobuli van de sublinguale speekselklier, pars polystomatica, zijn kleiner en komen meer verspreid voor. Daarenboven hebben ze aparte uitmondingen in kleine individuele sublinguale afvoergangen tussen de tong en de mandibula (laterale sublinguale recessus) (Dunning, 2003). De mandibulaire speekselklier, mediaal van de ventrale rand van de parotisklier en caudaal van m. masseter, ligt zoals hoger vermeld in een gemeenschappelijk kapsel met de pars monostomatica van de sublinguale speekselklier (Walker, 1982; Langley-Hobbs, 2014). Bij honden liggen de speekselklieren in het gemeenschappelijke kapsel tussen de linguofaciale en maxillaire vene terwijl bij katten de venen samenkomen op de laterale zijde van de klieren (Dunning, 2003). De mandibulaire afvoergang vertrekt zowel bij de hond als de kat aan de rostromediale zijde van de respectievelijke klier en loopt parallel met de ductus sublingualis. Beide lopen verder naar rostraal tussen de tongspieren naar de mondbodem. Daar monden ze in de meeste gevallen via afzonderlijke openingen uit in een gezamenlijke papil, namelijk de sublinguale karunkel, lateraal van het tongfrenulum (Walker, 1982; Dunning, 2003; Langley-Hobbs, 2014). Bij sommige honden is er een kleine sublinguale papil naast de mandibulaire papil op te merken (Peeters, 1991).

Walker (1982) wees erop om een duidelijk onderscheid te maken tussen de mandibulaire speekselklier (gelobuleerde structuur) en de lymfeknopen (kleiner en gladder) in deze regio. Effectief blijkt dit in praktijk niet altijd zo eenvoudig. In deze casus was er immers tijdens de chirurgie ook onduidelijkheid over de aard van de verschillende weefsels. Naast de mandibulaire speekselklier werden ook de lymfeknopen verwijderd, gezien het veranderde aspect van de lymfeknopen.

De waarschijnlijkheidsdiagnose van een ranula werd bij deze kat gesteld op basis van de anamnese en het klinisch onderzoek. De differentiaaldiagnosen voor een sublinguale zwelling zijn ranula, cyste, abces, vreemd voorwerp met granuloomvorming, hematoom, oedeem secundair aan een allergische reactie en neoplasie (Kiefer en Davis, 2007; Samanta, 2012). Gezien de zwelling zacht, fluctuerend en niet-pijnlijk was, was een sublinguale sialocoele of cyste het meest waarschijnlijk. Een neoplasie kan soms echter ook zacht en fluctuerend zijn (Radlinsky, 2013). Om zekerheid te bekomen omtrent de inhoud van de sublinguale zwelling wordt deze idealiter steriel geaspireerd. Macroscopisch wordt bij een sialocoele een heldere, mucoïde, goudgele vloeistof verwacht, eventueel met bloedbijmenging (Kiefer en Davis, 2007; Langley-Hobbs, 2014). Het cytologisch beeld van een sialocoele-aspiraat is meestal celarm (Kiefer en Davis, 2007; Langley-Hobbs, 2014). Secretorische epitheelcellen, gevacuoliseerde macrofagen, rode bloedcellen en/of neutrofielen zijn cellen die kunnen voorkomen. Afhankelijk van het stadium, acuut of chronisch, zijn er respectievelijk meer neutrofielen of hematoïdine kristallen. Deze laatste komen vrij bij de afbraak van oude, rode bloedcellen en duiden op voorgaande bloeding(en) (Allison en Maddux, 2008; Dietens et al., 2011). Een bijkomende "periodic acid-Schiff" (PAS)-kleuring, een specifieke mucuskleuring, kan uitgevoerd worden om de aanwezigheid van speeksel te bevestigen (Radlinsky, 2013). Speekselkliercysten en sommige sublinguale neoplasieën kunnen gelijkaardige bevindingen geven op cytologie en PAS-kleuring. Histopathologisch onderzoek blijft aangewezen om een definitieve diagnose te stellen (Radlinsky, 2013).

Voor de behandeling van sublinguale sialocoeles bij katten zijn er verschillende opties zoals drainage van de sialocoele, injectie met cauteriserende/anti-inflammatoire producten, marsupialisatie of het verwijderen van de betrokken speekselklieren. Het is ook mogelijk om enkele van de bovenstaande technieken te combineren (Kiefer en Davis, 2007; Radlinsky, 2013; Langley-Hobbs, 2014). Drainage van de ranula als enige behandeling leidt in veel gevallen tot recidieven (Kiefer en Davis, 2007; Kilic, 2009; Langley-Hobbs, 2014). Zowel herhaaldelijke drainage als injectie met cauteriserende of anti-inflammatoire producten pakt de oorzaak van de sialocoele niet aan. Daarenboven kunnen ze leiden tot abcedatie en fibrose, waardoor de daaropvolgende chirurgie bemoeilijkt wordt (Saifzadeh 2004; Radlinsky, 2013).

Marsupialisatie is een chirurgische techniek die typisch toegepast wordt bij sublinguale sialocoeles om de drainage van speeksel in de mondholte te bekomen (Dunning, 2003). De gesloten holte wordt geconverteerd naar een open zakje door een gedeelte van de sialocoelewand weg te snijden en de granulatieweefselrand aan de mondmucosa te hechten (Dunning, 2003, Kiefer en Davis, 2007; Radlinsky, 2013). Gezien het gering aantal beschreven casussen van sublinguale sialocoeles bij katten, zijn er maar weinig gegevens omtrent de prognose na marsupialisatie. In een retrospectieve studie van Kiefer en Davis (2007) bleek er geen recidief te zijn bij twee katten die louter met marsupialisatie werden behandeld. Theoretisch bestaat de mogelijkheid op recidief van de ranula wanneer de geopende sialocoele zich opnieuw sluit (Kilic, 2009). Hoewel marsupialisatie van de sublinguale sialocoeles als monotherapie effectief kan zijn, wordt er in de literatuur bij ranulae bij honden aangeraden om de veroorzakende speekselklier(en) ook te verwijderen (Kiefer en Davis, 2007; Radlinsky, 2013). De kans op recidief wordt zo tot een minimum beperkt. Bij de kat van de voorliggende casus werd een combinatie van marsupialisatie en sialoadenectomie van de mandibulaire en sublinguale speekselklier uitgevoerd. Beide speekselklieren worden steeds samen verwijderd omdat de sublinguale speekselklier nauw verbonden is met de mandibulaire alsook met zijn afvoergang. Verwijdering van de ene zou immers schade kunnen toebrengen aan de andere (Radlinsky, 2013; Langley-Hobbs, 2014). Postoperatieve xerostomia (droge mond) wordt bij de kat of de hond niet beschreven, zelfs niet na bilaterale verwijdering van mandibulaire en sublinguale speekselklieren, vermoedelijk omdat er zelfs dan nog steeds meerdere speekselklieren aanwezig zijn. Als alternatief was de combinatie van speekselklierexcisie met eenvoudige drainage van de sialocoele ook mogelijk geweest. Deze techniek is beschreven bij honden (Radlinsky, 2013).

Er zijn twee methoden bij de hond en de kat voor het uitvoeren van sialoadenectomie van de mandibulaire en sublinguale speekselklier. Enerzijds is er de traditionele, laterale benadering (Lane, 2012), die toegepast werd in deze casus. Hierbij wordt het speekselklierpakket net caudaal van de nervus lingualis geligeerd. Anderzijds is er de ventrale benadering waarbij het volledige klierpakket verwijderd kan worden tot juist ventraal van de sublinguale karunkel (Ritter et al., 2007; Lane, 2012).

In een studie van Lane (1994) werden 166 honden met faryngeale, cervicale of sublinguale sialocoeles en zeven katten met sublinguale sialocoeles opgenomen (Lane, 2012). Vier katten werden behandeld via de laterale en drie via de ventrale benadering. In alle zeven gevallen werden geen recidieven of complicaties beschreven. Bij de 166 honden werd 7% recidieven of persisterende sialocoeles opgemerkt na een correct uitgevoerde laterale sialoadenectomie. Met de ventrale benaderingstechniek (60 operaties) werden geen recidieven of persisterende sialocoeles beschreven (Lane, 1994; Ritter et al., 2006). De toegangsruimte gevormd door de caudale rand van de mandibula, de vena linguofacialis en vena jugularis is nauw bij de laterale benadering. Hierdoor is de dissectie van het speekselklierpakket tussen de tongspieren gecompliceerd. De meest rostrale lobuli van de pars polystomatica van de sublinguale speekselklier blijven bijna onvermijdelijk achter. Indien deze de oorzaak zijn van de sialocoele, wat bij deze recidieven ook het geval was, persisteert de sialocoele (Lane, 2012). Bij de ventrale techniek wordt de gehele klier blootgelegd en verwijderd. Dit is de reden waarom er bij het mislukken van de laterale techniek, de ventrale techniek een goede oplossing biedt (Lane, 2012). De nadelen van deze laatste techniek zijn een invasievere chirurgie, een grotere incisie en een langere operatieduur (Lane, 2012).

Complicaties na sialoadenectomie bij honden zijn weinig frequent (Tobias, 2010). Het recidiveren van mucocoeles en seroomvorming zijn het meest voorkomend (Radlinsky, 2013). Oorzaken van recidieven van sialocoeles zijn: behandeling van de verkeerde zijde, onvoldoende wegname van speekselklierweefsel en het wegnemen van de lymfeknopen in plaats van de klieren door bijvoorbeeld een beperkte anatomische kennis (Lane, 2012; Radlinsky, 2013). In een studie van Ritter et al. (2006) waarbij honden met ventrale sialoadenectomie werden behandeld, trad er in 17% van de gevallen seroomvorming op. Een seroom kan zich vormen door de ontstane dode ruimte en de beweeglijke regio ter hoogte van de incisie (Ritter et al., 2006; Radlinsky, 2013). Drainage of aspiratie ervan is niet nodig, tenzij de patiënt er last van ondervindt. Immers, een seroom resorbeert normaliter 10 tot 14 dagen postoperatief uit zichzelf (Lane, 2012; Radlinsky, 2013). Speekselklierexcisie is een propere operatie. Het respiratoire of gastro-intestinale stelsel wordt immers niet geopend. Een normale, aseptische operatietechniek is dan meestal ook voldoende om infecties te vermijden (Lane, 2012; Radlinsky, 2013). In de literatuur zijn er geen gevallen bij honden of katten beschreven van abcedatie na speekselklierexcisie (Lane, 2012). In deze casus werd de antibioticatherapie postoperatief gedurende tien dagen verdergezet.

Retrospectief gezien was er geen indicatie voor zo'n lange antibioticabehandeling. Profylactisch antibioticumgebruik om de kolonisatie van bacteriën te voorkomen zou zelfs niet nodig geweest zijn. Ter hoogte van de mond verzorgt speeksel immers een goede aspecifieke afweer (Soukup, 2009) en sialoadenectomie is een propere operatie.

Bij voorkeur worden de verwijderde weefsels histopathologisch onderzocht ter uitsluiting van neoplasie als oorzaak van de sialocoele (Radlinsky, 2013). Ook in deze casus werden de verwijderde weefsels onderzocht. Aan de speekselklieren en afvoergangen waren er geen abnormaliteiten. Ter hoogte van het dak van de ranula werden fistelgangen met een uitgesproken pyogranulomateuze ontsteking opgemerkt. Deze ontstonden vermoedelijk ten gevolge van automutilatie wanneer de kat op de ranula beet. Verder bleek dat er naast speekselklieren ook lymfeknopen werden verwijderd. In de lymfeknopen werden er naast lymfoïde hyperplasie met vorming van kiemcentra, nesten van mastcellen aangetroffen. De lymfoïde hyperplasie is allicht het rechtstreekse gevolg van de ontstekingsreactie ter hoogte van de ranula, terwijl de aanwezigheid van clusters mastcellen in de lymfeknoop wordt aanschouwd als een toevalsbevinding, aangezien er geen oorzakelijk verband met sialocoeles bestaat.

De prognose na de chirurgische behandeling van een sialocoele is uitstekend, op voorwaarde dat een juiste diagnose wordt gesteld en dat er een accurate en volledige speekselklierexcisie wordt uitgevoerd (Radlinsky, 2013). De prevalentie van recidieven na sialoadenectomie is laag, namelijk ≤ 7% (Lane 1994; Dunning 2003), tenminste als de betrokken speekselklieren volledig verwijderd werden. Bij de kat uit de huidige casus werd op het controlebezoek, vijf maanden na de ingreep, geen nieuwe ranulavorming vastgesteld.

CONCLUSIE

Een ranula bij een kat is een zeldzaam voorkomende aandoening. De waarschijnlijkheidsdiagnose kan gesteld worden op basis van de anamnese en het lichamelijk onderzoek. Een histopathologisch onderzoek blijft echter noodzakelijk om een cyste of neoplasie uit te sluiten. De prognose na sialoadenectomie is zeer goed. In de literatuur wordt er geen recidief gerapporteerd. Het aantal beschreven gevallen bij katten behandeld met sialoadenectomie is echter beperkt.

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Oproep

FACULTEIT DIERGENEESKUNDE
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EPI CENTRUM
Epilepsie bij hond en kat
Faculteit Diergeneeskunde - Universiteit Gent

Therapie-resistente idiopathische epilepsie bij de hond: en nu?

Patiënten gezocht voor onderzoek naar de doeltreffendheid van rTMS

Ongeveer één derde van de honden met idiopathische epilepsie heeft een slechte aanvalscontrole met de ingestelde medicamenteuze behandeling (aan de hoogste dosis en/of aan de juiste serumconcentraties). Voor deze therapie-resistente of refractaire honden hebben we vandaag de dag, jammer genoeg, geen behandelingsopties meer ter beschikking die hun doeltreffendheid reeds hebben bewezen.

Naar analogie met de humane geneeskunde willen we bij deze patiëntengroep graag het anti-epileptisch effect van **repetitieve transcraniële magnetische stimulatie** (rTMS) nagaan. Dit is een niet-pijnlijke, niet-invasieve manier van neurostimulatie en wordt bij de mens in wakkere toestand uitgevoerd. Bij honden is echter een lichte sedatie aangewezen (hond moet gedurende 1 uur stilliggen). Uit humane studies is gebleken dat magnetische stimulatie van de hersenen bij bepaalde patiënten kan leiden tot een betere aanvalscontrole.

Dit is de eerste grootschalige studie die zal uitgevoerd worden bij de hond wereldwijd.

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Two cases of feline pyothorax: medical versus surgical treatment and associated challenges

Twee gevallen van feliene pyothorax: medicamenteuze versus chirurgische behandeling en geassocieerde uitdagingen

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ABSTRACT

Pyothorax is a rare disease in cats. Underlying causes and treatment recommendations vary greatly between cases. In this case series, the management of two challenging cases of feline pyothorax is discussed. In the first case, a nine-year-old female spayed European shorthair cat with pyothorax caused by *Bacteroides fragilis* is described. At the time of presentation, she was diagnosed with feline immunodeficiency virus as well. The pyothorax was succesfully managed medically. Unfortunately, the cat relapsed after three months and the owner elected euthanasia. The second case involved a ten-year-old male castrated British shorthair cat with identification of filamentous bacteria on pleural fluid cytology. This cat underwent surgical intervention because thoracic drainage was very difficult. Eventually, he recovered well and did not relapse up to two months postoperatively. The challenges in the decision process and treatment complications are discussed.

SAMENVATTING

Pyothorax is een aandoening die niet vaak voorkomt bij katten. De onderliggende oorzaken en behandelingskeuze variëren sterk naargelang het geval. In het voorliggende artikel worden twee uitdagende casussen van pyothorax bij de kat besproken. In de eerste casus wordt een vrouwelijke, gesteriliseerde Europese korthaar van negen jaar met pyothorax ten gevolge van *Bacteroides fragilis* besproken. Bij presentatie van de kat op de Faculteit Diergeneeskunde (UGent) werd bij het dier bovendien het feliene immunodeficiëntie virus gediagnosticeerd. De pyothorax werd succesvol behandeld met medicamenteuze therapie, maar de kat herviel drie maanden nadien en werd geëuthanaseerd. In de tweede casus wordt een mannelijke, gecastreerde Britse korthaar van tien jaar beschreven, waarbij op het cytologisch onderzoek van de pleurale effusie filamenteuze bacteriën geïdentificeerd werden. De kat onderging een chirurgische ingreep, herstelde uiteindelijk goed en vertoonde geen tekenen van recidief tot twee maanden postoperatief. De uitdagingen bij het maken van belangrijke beslissingen en de complicaties tijdens de behandeling worden besproken.

INTRODUCTION

Pyothorax is characterized by an accumulation of septic exudate in the thoracic cavity (Ettinger and Feldman, 2010). Most commonly, oropharyngeal flora is isolated in the pleural fluid (Walker et al., 2000; Demetriou et al., 2002). While infection of the thoracic cavity through penetrating bite wounds or parapneumonic spread after aspiration of oropharyngeal flora, e.g. after dental procedures, seem to occur most often in cats, the underlying cause remains unknown in 30-65% of cases (Waddell et al., 2002; Barrs et al., 2005; MacPhail, 2007; Barrs and Beatty, 2009a). The treatment approach of pyothorax remains controversial, but can be broadly divided into two groups: medical and/or surgical management. Medical treatment usu-

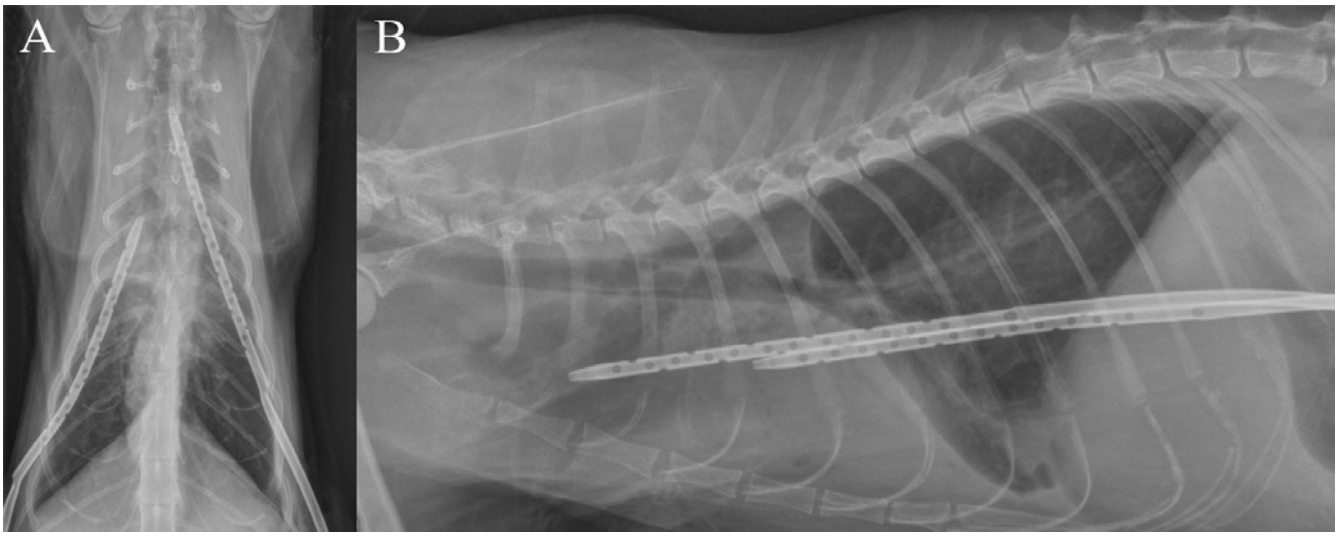
ally includes broad spectrum antibiotic treatment and thoracic drainage through thoracostomy tubes and often provides a good outcome in uncomplicated cases. In contrast, surgical treatment should be considered in cases that respond inadequately to the placement of thoracostomy tubes, in cases with isolation of filamentous bacteria in the pleural fluid, in cases of suspicion of or confirmed foreign body, and in cases with medical treatment failure after 2-7 days (Demetriou et al., 2002; Barrs et al., 2005; Monnet, 2009; Boothe et al., 2010; Murphy and Papasouliotis, 2011a; Stillion and Letendre, 2015). In this article, two cases of feline pyothorax are reported, enabling a thorough discussion of the differences between medical and surgical management and several factors influencing the clinician's decision process. The cat described in the first case tested positive for feline immunodeficiency virus (FIV). The compromised immune status of the cat required special measures to overcome pleural infection. In the pleural fluid of the second case, filamentous bacteria were isolated, complicating the general approach due to the associated pyogranulomatous reaction. The isolation of filamentous bacteria is one of the most common indications for early surgical intervention in pyothorax.

CASE 1

A nine-year-old female spayed European shorthair cat was presented at the referring veterinarian with chronic complaints of dyspnea, weight loss and lethargy and complete anorexia for three days. Upon examination, the cat had a fever. Antibiotics and non-steroidal anti-inflammatory drugs were initiated, but without clinical improvement. The following day, a thoracocentesis was performed due to muffled lung sounds on auscultation. Thick, yellow fluid was aspirated and the cat was referred (day 0) to the Small Animal Department, Faculty of Veterinary Medicine (Ghent University) for further diagnostic work-up and treatment.

Upon admission, the cat reacted aggressively to manipulation. She was tachypneic (70/min) and presented with an inspiratory dyspnea without stridor. Lung auscultation was muffled ventrally on both sides, but more pronounced on the left hemithorax. The remaining vital parameters were within normal limits. The cat showed mild muscle atrophy and severe dental plaque, tartar and gingivitis. The left eye showed remnants of an infection with feline herpes virus (FHV) as a kitten. The cat was kept strictly indoors, but the other cat in the household was allowed to go outside, and both cats did not get along very well.

The cat was admitted to the intensive care unit and supplemented with humidified oxygen in an oxygen cage. Quick in-house cytological evaluation of the pleural fluid provided by the referring veterinarian suggested pyothorax, based on the macroscopic appearance of the effusion and the presence of degenerated neutrophils and suspicion of intracellular bacteria on cytology. An intravenous (IV) catheter was placed and the cat was sedated with butorphanol (Dolorex®, Intervet International BV, Boxmeer, the Netherlands; 0.4 mg/kg IV), dexmedetomedine (Dexdomitor®, Orion Corporation, Espoo, Finland; 3 µg/kg IV) and midazolam (Dormicum®, NV Roche SA, Brussels, Belgium; 0.2 mg/kg IV). Afterwards, propofol (Propovet Multidose®, Zoetis Belgium SA, Louvain-la-Neuve, Belgium) was administered IV to effect, to allow placement of bilateral thoracic drains (Surgivet® Pneumothorax Set (10 Fr); Smiths Medical ASD Inc., Minnesota, United States of America). About 290 mL of pleural effusion was removed and correct positioning of the chest tubes was radiographically evaluated (Figures 1A and 1B). Thoracic radiographs did not reveal significant changes of the lung



Figures 1A and B. Control radiographs after the placing of bilateral thoracic drains in the cat of the first case. The right drain ends at the level of the fifth rib and was advanced slightly further afterwards. The left drain correctly ends at the level of the second rib. There is a moderate amount of pleural effusion present, characterized by the rounded lung lobes and the soft tissue opacity silhouetting with the heart.

Table 1. Blood results of both cases at presentation (day 0).

	Case 1	Reference values case 1	Case 2	Reference values case 2
Erythrocytes	**5.66**	6.54 – 12.2 x 10^{12}/L	**4.44**	5.00 – 10.00 x 10^{12}/L
Hematocrit	**23.1**	30.3 – 52.3 %	**22.9**	30.0 – 45.0 %
Hemoglobin	**8.1**	9.8 – 16.2 g/dL	10.7	9.0 – 15.1 g/dL
MCV	40.8	35.9 – 53.1 fL	51.6	41.0 – 58.0 fL
MCH	**14.3**	21.2 – 25.9 pg	**24.2**	12.0 – 20.0 pg
MCHC	35.1	28.1 – 35.8 g/dL	-	29.0 – 37.5 g/dL
RDW	23.1	15 – 27 %	20.9	17.3 – 22.0 %
Leukocytes	11.75	2.87 – 17.02 x 10^{9}/L	**38.01**	5.50 – 19.50 x 10^{9}/L
Neutrophils	**0.88**	1.48 – 10.29 x 10^{9}/L	**33.59**	2.50 – 12.50 x 10^{9}/L
Lymphocytes	**8.40**	0.92 – 6.88 x 10^{9}/L	1.00	0.40 – 6.80 x 10^{9}/L
Monocytes	**2.42**	0.05 – 0.67 x 10^{9}/L	1.58	0.15 – 1.70 x 10^{9}/L
Basophils	0.03	0.01 – 0.26 x 10^{9}/L	0.10	0.00 – 0.10 x 10^{9}/L
Eosinophils	**0.02**	0.17 – 1.57 x 10^{9}/L	**1.74**	0.10 – 0.79 x 10^{9}/L
Platelets	**19**	151 – 600 x 10^{3}/µL	**835**	175 – 600 µL x 10^{3}
Reticulocytes	**1.1**	3 – 50 x 10^{3}/µL	49.5	10 – 110 µL x 10^{3}
Urea	**14.3**	5.7 – 12.9 mmol/L	8	5.7 – 12.9 mmol/L
Creatinine	67	71 – 212 µmol/L	69	71 – 212 µmol/L
Total protein	66	57 – 89 g/L	60	57 – 89 g/L
Albumin	24	23 – 39 g/L	**18**	23 – 39 g/L
Globulin	42	28 – 51 g/L	42	28 – 51 g/L
ALT	21	12 – 130 U/L	101	12 – 130 U/L
ALP	14	14 – 111 U/L	37	14 – 111 U/L
Total bilirubin	-	-	**77**	0 – 15 µmol/L
Glucose	6.8	3.95 – 8.84 mmol/L	7.24	3.95 – 8.84 mmol/L
Sodium	**149**	150 – 165 mmol/L	161	150 – 165 mmol/L
Potassium	4	3.5 – 5.8 mmol/L	4.7	3.5 – 5.8 mmol/L
Chloride	-	-	122	112 – 129 mmol/L

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; ALT = serum alanine transferase activity; ALP = serum alkaline phosphatase activity.

field. The aspirated fluid grossly appeared yellow and opaque with large amounts of flocculent material. Pleural fluid cytology revealed a high cellularity, predominantly consisting of severely degenerated neutrophils and macrophages. Many extracellular and intracellular rod-shaped bacteria were present, confirming the suspected pyothorax. Total protein and total nucleated cell count (TNCC) were not performed since cytological examination was sufficient for diagnosis. A sample of the pleural effusion was submitted for aerobic and anaerobic culture.

A complete blood count (CBC) and serum biochemistry profile were performed, showing mild, non-regenerative anemia, mild lymphocytosis, severe neutropenia, pseudothrombocytopenia and slightly elevated urea (Table 1). Blood smear revealed moderate neutrophilia with left shift. The neutropenia seen on CBC can be explained as a laboratory error, in which the automated cell counts probably miscounted band neutrophils as lymphocytes. Given that the cat belonged to a multi-cat household with a history of intercat aggression, additional testing for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) was performed (SNAP® COMBO FeLV Ag/FIV Ab TEST KIT, Idexx Laboratories Inc., Maine, United States of America); the cat tested positive for FIV.

During the evening, the respiratory rate decreased to acceptable limits (36 to 44 breaths/min), the dyspnea resolved and the oxygen supplementation was stopped. The cat received maintenance fluid therapy (Sterofundin B®, B. Braun Melsungen AG, Melsungen, Germany; 70 mL/kg/day IV) as well as Hartmann's infusion (Vetivex Hartmann's Solution®, Dechra Limited, Staffordshire, United Kingdom; 35 mL/kg/day IV) for rehydration and to compensate for loss of fluids through thoracic drainage. The chest tubes were emptied every four hours and thoracic lavage with Hartmann's solution was performed every eight hours (initially 10 mL/kg, thereafter 20 mL/kg). Antibiotic therapy was initiated with amoxicillin-clavulanic acid (Augmentin®, GlaxoSmithKline Pharmaceuticals SA/NV, Waver, Belgium; 20 mg/kg q8h IV) and enrofloxacine (Baytril 2.5%®, Bayer SA/NV, Diegem, Belgium; 5 mg/kg q24h SC). Buprenorphine (Vetergesic®, Alstoe Limited, York, United Kingdom; 10 µg/kg q8h IV) was initiated for pain management.

On day 1, the cat remained mildly tachypneic but the lung sounds were less muffled. The general condition was improved, but the cat remained anorexic. Omeprazole (Losec®, AstraZeneca SA/NV, Brussels, Belgium; 1 mg/kg q24h IV) was initiated. The thoracic drains were still highly productive with a total of 34.6 mL/kg/day.

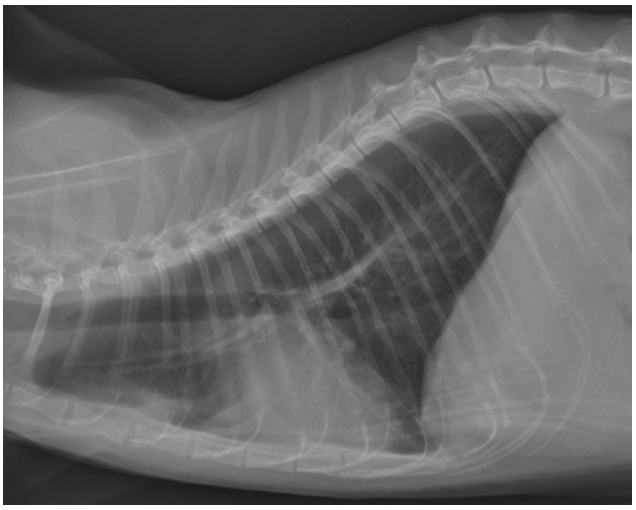


Figure 2. Right lateral thoracic radiograph of the European shorthair cat two weeks after discharge, showing marked improvement of the pleural effusion. There are some pleural fissure lines present, most likely indicating thickening of the pleura due to scar tissue.

Over the next days, the cat's respiratory pattern normalized. Repeated pleural fluid cytology revealed decreasing numbers of neutrophils, macrophages and intracellular bacteria. By day 3, chest tube production had decreased to 6.4 mL/kg/day and bacteria were only rarely detected on pleural fluid cytology. Unfortunately, the cat's good clinical response coincided with more aggressive behavior, which was an important factor in the cat's further management. The cat was sedated with butorphanol (Dolorex®; 0.3 mg/kg IV) and both thoracic drains were removed. Because the cat still showed poor appetite, a naso-esophageal feeding tube was placed and tube feeding was started. On day 4, bacteriological culture of the pleural fluid came back positive for *Bacteroides fragilis,* sensitive to amoxicillin-clavulanic acid, and enrofloxacine was discontinued. Biochemistry was repeated and revealed normalization of the serum urea concentration. The cat regained appetite and was discharged on day 5 with amoxicillin-clavulanic acid (Kesium®, SOGEVAL, Laval, France; 20 mg/kg q12h PO) for a minimum of four weeks.

Recheck thoracic radiographs were advised one week after discharge, but the cat was presented two weeks later. At this point, she was still on antibiotics and showed no clinical signs of recurrence. She was eating well and had no respiratory complaints. On physical examination, there was neither tachypnea nor dyspnea. Auscultation of the lung field was normal, but a systolic heart murmur of 2/6 was detected at the level of the sternum. Due to the cat's uncooperative behavior, only one lateral view was performed and revealed pleural fissure lines, most likely indica-ting thickening of the pleura (scar tissue) associated with the pyothorax (Figure 2). The antibiotics were continued until the next control visit two to three weeks later. Echocardiography was offered, but declined by the owner.

Further follow-up was performed by the referring veterinarian. The antibiotic therapy was prolonged for several additional weeks and the cat remained clinically stable. Unfortunately, three months after discharge, pyothorax recurred. The owners decided not to treat again and the cat was euthanized.

CASE 2

A ten-year-old male castrated British shorthair cat was presented to the referring veterinarian for detartration. Afterwards, he was lethargic and completely anorexic for two days. He remained partially anorexic

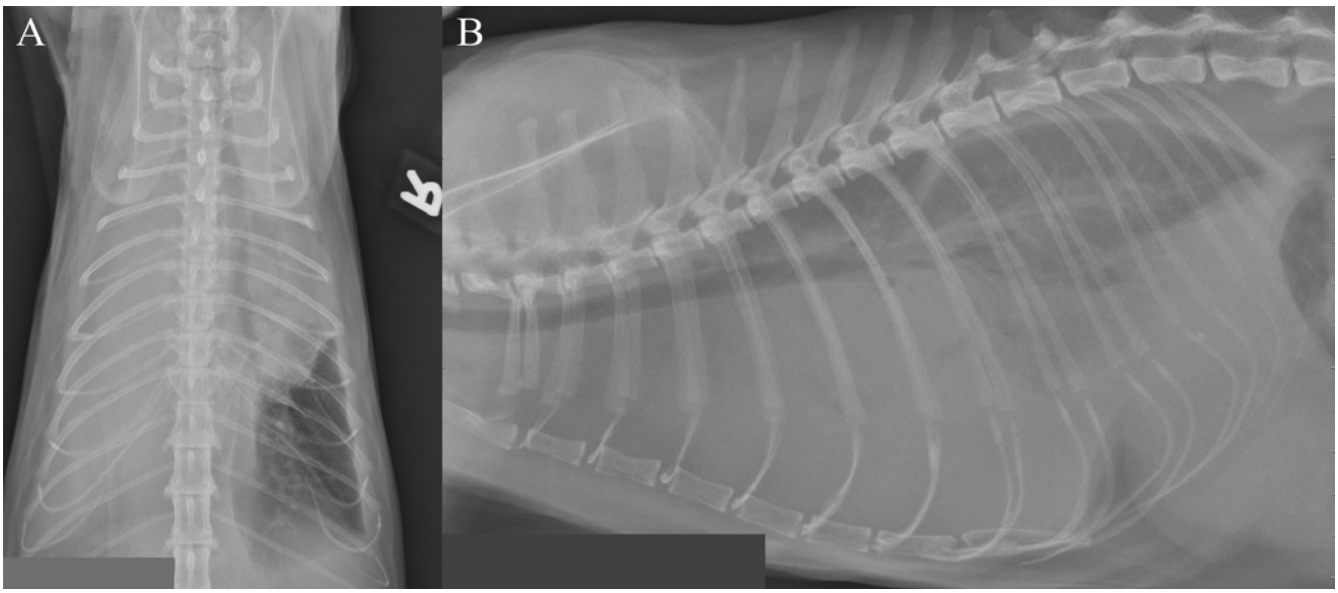


Figure 3. A. Right lateral and B. dorsoventral thoracic radiographs of the cat of the second case, showing very severe pleural effusion, more pronounced in the left hemithorax. The cardiac silhouette is masked by the soft tissue opacity of the free fluid and the trachea is displaced dorsally. The lungs are severely displaced caudodorsally on the lateral projection. There is an alveolar pattern present in the right cranial lung lobe.

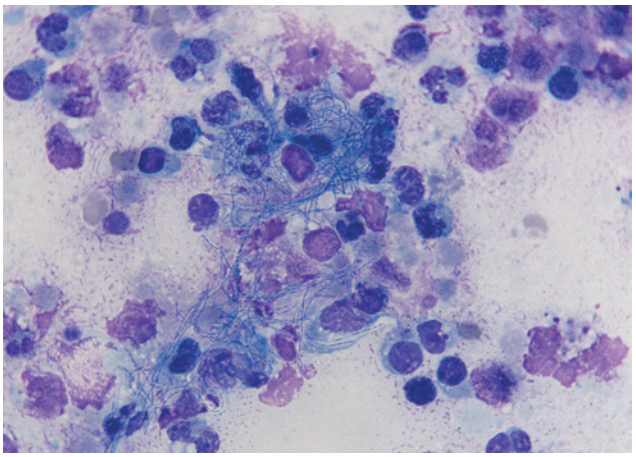


Figure 4. Hematoxylin and eosin stain of the pleural fluid of the British shorthair cat, showing large clusters of filamentous bacteria and large amounts of degenerated neutrophils and macrophages.

over the following three weeks and lost 600 grams. At that point, tachypnea, dyspnea and a fever of 40°C were identified. The cat received tolfenaminic acid (Tolfedine® 4%, Vétoquinol BV, 's-Hertogenbosch, Belgium; 4 mg/kg IM) and cefovecin (Convenia®, Zoetis Belgium SA, Louvain-La-Neuve, Belgium; 8 mg/kg IM), without improvement. Two days later, thoracic radiographs revealed the presence of mainly left-sided pleural effusion. Only a small amount of thick fluid was removed by thoracocentesis. Therefore, the cat was referred (day 0) to the Small Animal Department of the Faculty of Veterinary Medicine (Ghent University).

Physical examination revealed tachypnea with severe dyspnea – mainly inspiratory – without stridor, tachycardia and lethargy. Muffled lung- and heart sounds were auscultated, especially on the left hemithorax. The cat had pale pink mucous membranes with normal capillary refill time, and femoral pulses were weak. The blood pressure (BP), measured with the Doppler ultrasonic technique, was within normal limits (96 mmHg). CBC and serum biochemistry revealed mild, non-regenerative anemia, moderate leukocytosis, consisting of moderate neutrophilia and mild eosinophilia, mild thrombocytosis, moderate hypoalbuminemia and marked hyperbilirubinemia (Table 1). After stabilization with humidified oxygen delivered through a face mask, thoracic radiographs showed severe pleural effusion with asymmetric distribution, more pronounced in the left hemithorax. An alveolar pattern in the left cranial lung lobe was suspected, but this was to be re-evaluated after removal of the pleural fluid (Figures 3A and 3B).

Bilateral thoracocentesis was performed, removing 50 mL of thick, yellow pus containing flocculent material. On cytology, the pleural fluid showed a high cellularity and contained mostly degenerate neutrophils with smaller numbers of macrophages and areas of necrotic material. These findings were consistent with a purulent exudate. Large clusters of filamentous

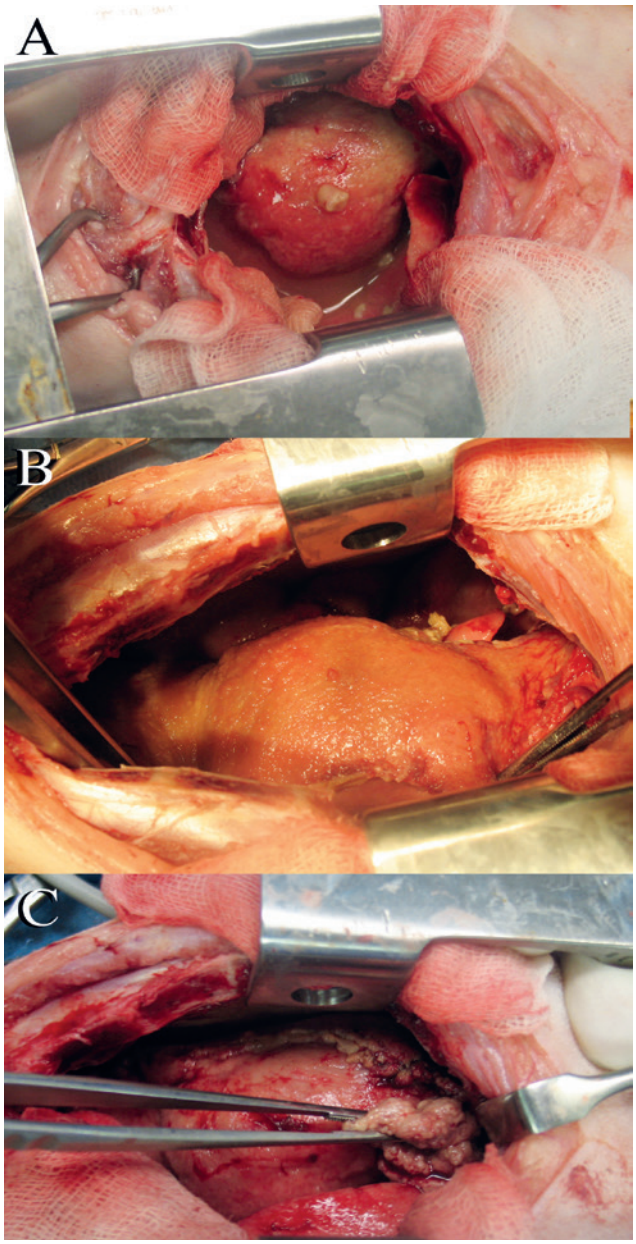


Figure 5. Intraoperative images showing A. a large amount of thick, pleural effusion with flocculent and fibrinous material B. left hemithorax with collaps of the lung lobes, covered with fibrinous material and C. a severely reactive and thickened mediastinum.

organisms confirmed the diagnosis of pyothorax (Figure 4). The cat was sedated with fentanyl (Fentadon®, Dechra, Bladel, the Netherlands; 5 µg/kg IV) and a local intercostal block was performed with lidocaine (Xylocaïne® 2%, AstraZeneca BV, Zoetermeer, the Netherlands; 4 mg/kg) to allow the placement of two thoracic drains. About 200 mL of red-brownish fluid with flocculent material was removed over the next ten hours, but drainage remained difficult. The cat was hospitalized and was started on crystalloid infusion (Vetivex Hartmann's Solution®; 90 mL/kg/24h), amoxicillin-clavulanic acid (Augmentin®; 20 mg/kg q8h IV) and enrofloxacine (Baytril 2.5%®; 5 mg/kg q24h IV). For analgesia, buprenorphine (Vetergesic®; 20 µg/kg q6h IV) was administered.

Table 2. Follow-up blood results during the hospitalization of case 2.

	Day 2	Day 3	Day 4	Day 5	Day 7	Day 9	Reference values
Erythrocytes	**3.37**	**4.36**	5.26	5.42	5.01	5.26	5.00 – 10.00 x 10^{12}/L
Hematocrit	**17.6**	**22.6**	**27.3**	**28.6**	**26.0**	**26.5**	30.0 – 45.0 %
Hemoglobin	**6.5**	9.6	9.5	10.4	9.7	9.7	9.0 – 15.1 g/dL
MCV	52.2	51.9	51.9	52.8	51.8	50.4	41.0 – 58.0 fL
MCH	19.3	**22.1**	18.2	19.3	19.3	18.4	12.0 – 20.0 pg
MCHC	37.0	-	35.0	36.5	37.3	36.5	29.0 – 37.5 g/dL
RDW	19.6	20.0	18.7	19.1	19.4	20.3	17.3 – 22.0 %
Leukocytes	**60.3**	**56.48**	**36.99**	**27.76**	**33.34**	**29.94**	5.50 – 19.50 x 10^9/L
Neutrophils	**54.72**	**50.22**	**31.91**	**24.41**	**30.03**	**20.37**	2.50 – 12.50 x 10^9/L
Lymfocytes	0.41	0.48	1.03	0.88	0.51	0.72	0.40 – 6.80 x 10^9/L
Monocytes	**2.75**	**3.94**	**2.20**	1.22	1.48	1.24	0.15 – 1.70 x 10^9/L
Basophils	**0.23**	0.09	**0.20**	**0.16**	**0.22**	**0.19**	0.00 – 0.10 x 10^9/L
Eosinophils	**2.19**	**1.76**	**1.65**	**1.09**	**1.10**	**2.42**	0.10 – 0.79 x 10^9/L
Platelets	403	**642**	524	575	434	**783**	175 – 600 x 10^3/µL
Reticulocytes	41.1	33.5	40.8	82.7	34.0	58.7	10 – 110 x 10^3/µL
Urea	-	-	-	-	2.5	-	5.7 - 12.9 mmol/L
Creatinine	69	74	-	-	73	-	71 – 212 µmol/L
Total protein	**43**	**56**	-	-	65	-	57 – 89 g/L
Albumin	**10**	**16**	**17**	-	**20**	**22**	23 – 39 g/L
Globulin	33	40	-	-	45	-	28 – 51 g/L
ALT	-	-	-	-	116	-	12 – 130 U/L
ALP	-	-	-	-	**135**	-	14 – 111 U/L
Total bilirubin	**63**	**62**	-	**46**	-	-	0 – 15 µmol/L
Glucose	-	-	-	-	7.43	-	3.94 – 8.83 mmol/L
Sodium	156	159	158	-	163	161	150 – 165 mmol/L
Potassium	4.3	**3.3**	3.7	-	-	4.5	3.5 – 5.8 mmol/L
Chloride	125	120	118	-	120	117	112 – 129 mmol/L
Phosphorus	-	-	1.49	1.71	-	-	1.00 – 2.42 mmol/L

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; ALT = serum alanine transferase activity; ALP = serum alkaline phosphatase activity.

The next day (day 1), the cat remained tachypneic with mild inspiratory dyspnea and muffled lung sounds. Because drainage through the thoracic drains was difficult and filamentous bacteria were seen on cytology, the cat underwent an exploratory sternotomy. He received fentanyl (Fentadon®, Dechra, Bladel, the Netherlands; 5 µg/kg IV) as premedication, and anesthesia was induced with alfaxalone (Alfaxan®, Jurox (UK) Limited, Worcestershire, United Kingdom; 3 mg/kg IV) and maintained with isoflurane (IsoFlo®, Zoetis Belgium SA, Louvain-La-Neuve, Belgium) vaporized in oxygen using a rebreathing system, combined with a constant rate infusion (CRI) of fentanyl (Fentadon®, Dechra, Bladel, the Netherlands; 5 µg/kg/h IV). Inspection of the pleural cavity showed a large quantity of thick, yellow fluid. In the left hemithorax, all lung lobes were collapsed and covered with fibrous material. The right hemithorax showed no collapsed lung lobes, but adhesions between the lung and the parietal pleura were present. The mediastinum was severely reactive and thickened and was removed for the most part (Figure 5). Samples for aerobic and anaerobic bacteriologic and histologic examination were taken and submitted to the lab. A new thoracic drain was placed and secured with a Chinese finger trap suture. An esophageal feeding tube was placed to ensure adequate nutritional support. Additionally, a fentanyl patch (Duragesic®, Janssen Pharmaceutica Products, L.P., New Jersey, United States of America) was placed onto shaved and cleaned skin to provide analgesia during hospitalization.

Thoracic radiographs were taken to ensure the correct placement of the esophageal feeding tube as well as the thoracic drain. Postoperatively, the cat suffered from severe hypotension (BP of 74 mmHg), and serum biochemistry revealed severe hypoalbuminemia (Table 2). He received two boluses of colloids (HAES-steril 6%®, Fresenius Kabi NV/SA, Schelle, Belgium; 2.5 mL/kg IV) and was placed on a CRI of colloids (HAES-steril 6%®; 0.8 mL/kg/h IV). Because the blood pressure (BP) did not improve, a CRI of norepinephrine (Levophed®, Hospira Benelux BVBA, Antwerp, Belgium; 0.1 µg/kg/min IV) was started. After gradually increasing the dose to 0.18 µg/kg/min, the BP increased to normal values (90-92 mmHg). The thoracic drain was emptied every four hours and a total of 68 mL (15.1 mL/kg/day) was collected on day 1. Buprenorphine was replaced by methadone

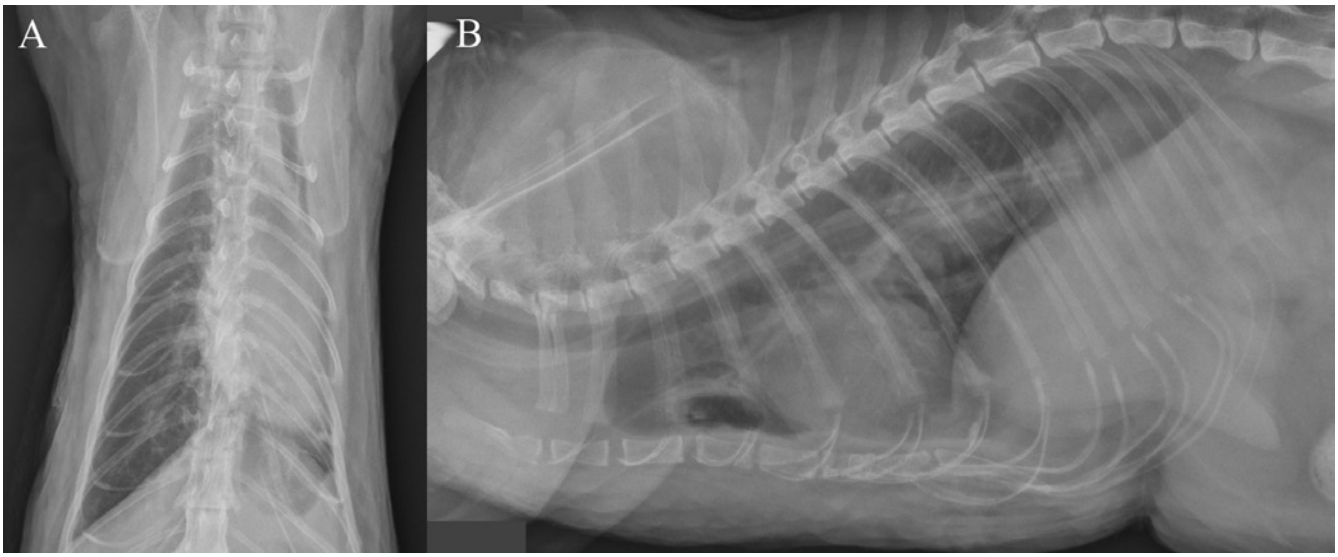


Figure 6A. Lateral and B. dorsoventral thoracic radiographs of the British shorthair cat on day 9. The radiographs show improvement of the atelectasis of the left cranial lung lobe, which had previously been seen. A left-shift of the mediastinum and the cardiac silhouette is still present due to remaining decreased volume of the left lung. A focal area of pleural effusion persists at the level of the left costodiaphragmatic angle.

(Comfortan®, Eurovet Animal Health BV, Bladel, the Netherlands; 0.3 mg/kg q4h IV) until sufficient activity of the fentanyl patch could be reached and meloxicam (Metacam®, Boehringer Ingelheim Vetmedica GmbH, Ingelheim/Rhein, Germany; 0.1 mg/kg SID SC) was added to the therapy.

On day 2, the BP decreased again (75-84 mmHg). Therefore, meloxicam was not repeated, and the CRI of norepinephrine was increased to 0.22 µg/kg/min. Blood examination revealed persistent hypoalbuminemia and more severe anemia (Table 3). A compatible whole blood transfusion was administered without complications. During the day, a total of 86 mL (20.2 mL/kg/day) of pleural effusion was collected. The neutrophils still showed signs of degeneration, but less than on the initial smears. The macrophages were strongly vacuolated. Only a few rod-shaped intracellular bacteria were seen, but no more chains of filamentous bacteria were present. Tube feeding was started in the evening. Methadone was discontinued, further therapy remained the same.

The next day (day 3), the hypoalbuminemia and anemia had slightly improved (Table 3). Because the cat developed hypokalemia, the Hartmann infusion was supplemented with potassium (Kali-Sterop®, Laboratoria STEROP NV; Brussels, Belgium; 30 mEq/L). The CRI of colloids and norepinephrine could be decreased gradually while maintaining normal BP, and both were discontinued eventually. The total amount of pleural effusion aspirated decreased to 28 mL (6.4 mL/kg/day).

On day 4, thoracic radiographs showed small remnants of pleural effusion, but an iatrogenic pneumothorax was caused, because the three way-stop valve got detached. Fortunately, this was quickly resolved. The hypokalemia normalized and the potassium supplementation was stopped. The hypoalbuminemia, anemia and leukocytosis kept on improving and total aspirated pleural fluid decreased to 18 mL (3.9 mL/kg/day) (Table 3). The pleural fluid had a lower cellularity than the days before and no more bacteria were seen. Despite the cytological presence of filamentous organisms, bacterial culture after three days of growth came back negative. Histological examination revealed reactive proliferations, most likely in response to bacterial antigens, with rod-shaped bacteria in a single area. No filamentous bacteria were seen and acid-fast stains were negative.

During the following days, the cat remained stable, but calm and anorexic. On day 5, the thoracic drain was removed and on day 6, the antibiotics were changed from intravenous to oral administration. Enrofloxacine (Xeden®, SOGEVAL, Laval, France; 5 mg/kg SID PO) and amoxicillin clavulanic acid (Clavubactin®, Le Vet BV, Oudewater, the Netherlands; 12.5 mg/kg BID PO) were administered. The Hartmann infusion was continued at maintenance rate. The next day (day 7), definitive bacterial cultures came back negative as well. It was decided to add trimethoprim-sulphadiazine (Tribrissen®, Intervet International BV, Boxmeer, the Netherlands; 15 mg/kg SID PO), because the clinical condition of the cat did not improve sufficiently, suggesting ongoing infection despite negative culture results. Afterwards, the cat became more active. Blood results improved further and thoracic radiographs were repeated on day 9, which showed improvement of the atelectasis of the left cranial lung lobe and little remaining pleural effusion (Table 2; Figures 6A and 6B).

The cat was discharged on oral antibiotics (enrofloxacine, amoxicillin clavulanic acid and thrimethoprim-sulphadiazine), and feeding through the esophageal feeding tube were continued at home. Fortunately, the cat quickly started eating spontaneously at home

and the esophageal feeding tube could be removed. Two months after discharge, the cat was active, had a good appetite and had already gained some weight. Recheck thoracic radiographs were taken by the referring veterinarian, which showed no more pleural effusion and further improvement of the atelectasis of the left lung lobes. Unfortunately, the cat was lost to further follow-up.

DISCUSSION

In this article, both medical and surgical treatments of pyothorax in cats are discussed based on the description of two different cases. It is obvious that the treatment approach should be adjusted according to each individual case. The first case showed no immediate indication for surgical intervention. Medical treatment was started and the cat rapidly responded. The second case was diagnosed with a more complex pyothorax from the beginning, characterized by inadequate drainage through thoracostomy tubes and cytological detection of filamentous bacteria. It was quickly decided that surgical exploration would be necessary to provide a good outcome. Not all cases of pyothorax are this clearly divided into those that need surgery and those that do not. Therefore, all cases of pyothorax should undergo thorough work-up, minimally consisting of thoracic radiographs after fluid drainage and cytological evaluation and bacteriological culture of the pleural fluid, to assess whether or not any complicating factors could be present (MacPhail, 2007).

Although in case 1, straightforward medical treatment of pyothorax is described, the treatment approach was in fact complicated by the FIV-positive status of the cat. An association between pyothorax and FIV-positive cats could be expected, given that both can be attained through bite wounds. Moreover, the immuosuppression caused by FIV might be a predisposing factor for bacterial infections, possibly leading to pyothorax. Currently, no direct relationship between pyothorax and FIV seems to be present. The prevalence of FIV in cats with pyothorax seems to be low (1.5%), although only 50 of 128 affected cats have actually been tested (Demetriou et al., 2002; Waddell et al., 2002; Barrs et al., 2005). Given that the cat was only diagnosed with FIV at presentation for pyothorax, it is difficult to assess the influence of FIV on the pleural space disease process. The anamnesis did not reveal any relevant medical history, but the acute phase of infection with FIV, characterized by transient sickness, often goes unnoticed by the owners. This suggests that at presentation, the cat was either in the subclinical phase of FIV-infection, undergoing progressive immunosuppression, or in the terminal phase, with the bacterial infection of the pleural space as an opportunistic infection (Sykes, 2014a).

The cat in this case responded well to conservative treatment, consisting of analgesia, thoracic drainage with lavage and antibiotic therapy. Initially, amoxicillin-clavulanic acid and enrofloxacine were administered. In light of increasing antibiotic resistance, it would have been better to initiate therapy with amoxicillin-clavulanic acid or amoxicillin only, given that the obligate anaerobic bacteria most frequently isolated from pyothorax in cats, are often susceptible to penicilline derivates (Walker et al., 2000; Demetriou et al., 2002; Barrs et al., 2005; Barrs and Beatty, 2009b). However, the veterinary clinicians in this case report take increasing antibiotic resistance into consideration through the use of de-escalation therapy, i.e. discontinuation of ineffective antibiotics after susceptibility testing or based on clinical response (Weese et al., 2015). In human medicine, de-escalation therapy has been successfully used in order to minimize antibiotic resistance and no negative impact on clinical condition has been seen (Gonzalez et al., 2013; Mokart et al., 2014). In the cat of this case report, the isolated bacteria were susceptible to amoxicillin-clavulanic acid and therefore, enrofloxacine was discontinued. By doing so, the development of antibiotic resistance might have been be minimized (Weese et al., 2015).

In the literature, there is no general consensus as to when thoracic drains are ideally removed in cases of pyothorax. Most commonly, drain removal is advised when fluid production is declining, cytological evidence of infection resolution is present and the patient is responding well clinically. Ideally, bacterial culture results should confirm appropriate antibiotic therapy prior to removal (Demetriou et al., 2002; Klainbart et al., 2007; Barrs and Beatty, 2009b; Marques et al., 2009; Murphy and Papasouliotis, 2011b). Several external factors took part in the decision to prematurely remove the drains in this case. The cat was difficult to manipulate, especially regarding thoracic drainage and lavage, and the owners were financially limited. Given that drain production was low, clinical response was good and significant cytological improvement of infection was seen, the thoracic drains were removed in spite of few bacteria still being present on cytology of the pleural fluid. In this case, the results of culture and susceptibility testing were not available yet, to confirm appropriate antibiotic treatment.

This case sets a good example for treating FIV-positive cats. Although these cats are more susceptible to opportunistic infections, they may be as capable as FIV-negative cats in fighting infections. However, it might be necessary to prolong antibiotic therapy or to treat more aggressively to enable full recovery (Hosie et al., 2009). In one case, a cat tested positive for FIV when being presented for recurrence of pyothorax (Demetriou et al., 2002). It can be suggested that there is a link between FIV and recurrence rates, but data supporting this belief are insufficient and recurrence is a known complication of pyothorax in cats, regardless of the immune status (Demetriou et

al., 2002; Waddell et al., 2002; Boothe et al., 2010). In the cat of this case report, the pyothorax relapsed three months after discharge, but it remains uncertain whether this can be attributed to the FIV-positive status of the cat.

In case two, a complex example of pyothorax in cats caused by filamentous organisms is described. Filamentous bacteria most commonly isolated in pyothorax include *Actinomyces* spp. and *Nocardia* spp. (Demetriou et al., 2002; Waddell et al., 2002; Barrs et al., 2005). Most commonly, *Nocardia* spp. are acid-fast whereas *Actinomyces* spp. are not. However, when the infecting strain of *Nocardia* is not acid-fast, differentiation between both may be difficult (Sykes, 2014b). Although *Nocardia* spp. are usually not difficult to isolate on most routine bacteriologic media (Saubolle and Sussland, 2003), the culture results came back negative, hence, definitive diagnosis and susceptibility testing were not possible in this case. In this case, acid-fast stains on the histological samples came back negative, but the cat was suspected to be infected with *Nocardia* spp. based on poor response to initiated antibiotic therapy. *Nocardia* spp. are gram-positive filamentous saprophytes that are found in dust, organic material and water. Infection occurs through inhalation or through inoculation of skin wounds. Nocardiosis is often seen as an opportunistic infection in immunocompromised patients (Malik et al., 2006; Sykes, 2014b). The cat in case 2 did not undergo FIV/FeLV-testing because he was strictly kept indoors and had no contact with other cats, making infection unlikely. There were no reasons to assume that the cat was immunocompromised. Typically, nocardiosis is divided into three clinical syndromes: cutaneous, pulmonary and disseminated nocardiosis (Malik et al., 2006; Sykes, 2014b). In pulmonary nocardiosis, infection most likely occurs through inhalation, and intra- and/or extrapulmonary masses are formed, possibly leading to pyothorax (Sykes, 2014b). In this case, it is important to acknowledge dental procedures as a possible underlying cause of pyothorax. In most cases of pyothorax, oropharyngeal flora is isolated. These bacteria can be aspirated during dental procedures (Demetriou et al., 2002; Barrs et al., 2005). This cat had recently undergone detartration, but it was difficult to confirm a causal relationship. *Actinomyces* spp. may form part of the normal oropharyngeal flora in cats, whereas *Nocardia* spp. do not (Walker et al., 2002; Barrs et al., 2005).

Pyothorax caused by filamentous organisms generally does not respond well to medical therapy because of the associated pyogranulomatous inflammatory response, complicating sufficient drainage of the pleural exudate (Sivacolundhu et al., 2001; Malik et al., 2006). In this case, drainage through thoracostomy tubes was indeed very difficult. Thoracic radiographs revealed an alveolar pattern, and computed tomography (CT) was proposed to the owners to provide more detailed information regarding the cause. The owners did not consent to this scan and decided on early exploratory surgery instead. The performed mediastinectomy was necessary to enable adequate thoracic drainage, which is one of the cornerstones of successful treatment of pyothorax (Piek and Robben, 2000; Rooney and Monnet, 2002; Boothe et al., 2010).

Postoperatively, the cat in case 2 did not recover without complications. The cat developed severe hypotension. In cats with pyothorax, this is most commonly seen due to the development of sepsis (Brady et al., 2002). Most common complications after thoracic surgery include pain and/or wound dehiscence and discharge. Postoperative hypotension has not been thoroughly described, but recovery after thoracic surgery seems to be more guarded for cats than for dogs (Moores et al., 2007; Tilson, 2016). In general, the mortality rates of cats and dogs undergoing thoracic surgery vary according to underlying disease, with an intermediate survival rate (60-70%) for cats and dogs undergoing thoracic surgery for pleural effusion (Bellenger et al., 1996; Tilson, 2016). Severe complications, including death, seem to be associated more often with the underlying cause than with the surgery itself (Moores et al., 2007). Postoperatively, the cat in case two was hemodynamically unstable and the prognosis did seem more guarded at that time point. Intensive treatment with fluid therapy and vasopressors was necessary to gradually restore normal blood pressure. Only after two days, the colloids and norepinephrine could be discontinued and the cat was able to maintain normal blood pressure. Meloxicam was administered for perioperative pain management, when blood pressure was normalized. The use of non-steroidal anti-inflammatory drugs in a hypotensive patient is contraindicated because of the increased risk of adverse reactions, such as renal injury. Such drugs should be used cautiously in the presence of hypotension (Berry, 2015; Hunt et al., 2015). Therefore, when the blood pressure lowered again later on, meloxicam was discontinued. Further, on day 4, iatrogenic pneumothorax was caused through detachment of the three-way stop valve while taking thoracic control radiographs. Luckily, there were no other injuries to the thoracic cavity, the intrathoracic air was quickly evacuated through the intact thoracic drain and the cat did not suffer from any consequences afterwards. The long-term presence of thoracostomy tubes is not without risk. It has been reported that cats with chest tubes develop a variety of complications in over half of cases, ranging from local irritation to nerve damage, hemorrhage and pneumothorax (Barrs et al., 2005).

It was difficult to assess appropriate antibiotic therapy in this case, because there was no culture or susceptibility testing to account for. When there is a suspicion of *Nocardia* spp., first-choice antibiotics consist of trimethoprim-sulfonamides (TMS), but these are often disguarded due to the potential side effects, such as nausea, anorexia and myelosuppression, which may occur when treating at high dosages

for a long period of time, which is usually required for the treatment of pyothorax (Malik et al., 2006; Sykes, 2014b). Further, different strains of *Nocardia* spp. respond to different types of antibiotics and a broad-spectrum approach is for that reason often initiated instead. Studies in cats have shown that antimicrobial resistance of the most common strains of *Nocardia* to fluoroquinolones and amoxicillin-clavulanic acid is high and therefore, both should not be recommended for treating nocardiosis (Malik et al., 2006; Govendir et al., 2011). While waiting for culture results, the antibiotic therapy of the case discussed above initially consisted of the combination of amoxicillin-clavulanic acid and enrofloxacine. Although this is not the first choice for treating nocardiosis, it is generally effective for the treatment of other bacteria causing pyothorax, including *Actinomyces* spp. (Sivacolundhu et al., 2001; Rooney and Monnet, 2002; MacPhail, 2007). Unfortunately, nocardiosis could never be confirmed. The culture results came back negative and repeated cultures were not performed as it was unlikely to yield positive results after treatment with broad-spectrum antibiotics for several days. The insufficient improvement of the clinical condition of the cat raised suspicion and after six days, TMS were added to the therapy. According to de-escalation therapy, amoxicillin-clavulanic acid and enrofloxacine should have been discontinued and replaced by TMS (Weese et al., 2015). The lack of culture and susceptibility testing in this case made it difficult to safely adjust antibiotic therapy. Therefore de-escalation therapy was not applied. Furthermore, it is difficult to attribute the progression of the clinical condition to the initiation of TMS, because clinical improvement of nocardiosis is often only seen after approximately seven days of antibiotic treatment (Sykes, 2014b).

After discharge, the cat recovered well and was treated with long-term antibiotics. The cat did not show any signs of recurrence two months after discharge. It is not clear for how long the antibiotic therapy was prolonged and if all antibiotics were continued long-term. Nocardiosis usually has a guarded long-term prognosis because of high recurrence rates (Malik et al., 2006). Data concerning the appropriate duration of antibiotic treatment of pyothorax caused by *Nocardia* spp. in cats is scarce. The total duration of antibiotic treatment should be three to six months, but it may be required for up to a year to prevent the patient from relapsing (Sivacolundhu et al., 2001; Malik et al., 2006; Yildiz and Doganay, 2006).

CONCLUSION

In this case series, one case of pyothorax that was medically managed and one case that required surgical treatment are described. These two cases show that treatment approach should always be assessed individually in order to provide a good outcome and that challenges occur in cats with pyothorax. Recurrence is a common complication of pyothorax, which was of significant importance in the first case, describing pyothorax in an immunocompromised patient. In this case, prolongation of antibiotic therapy should be advised. The second case involved filamentous bacteria, causing an intrathoracic pyogranulomatous inflammatory response. Antibiotic therapy should be long-term in this case as well. In both cases, there was no clear consensus as to when the antibiotic therapy could be safely terminated.

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The impact of antimicrobial use guidelines on prescription habits in fourteen Flemish small animal practices

De impact van advies omtrent het gebruik van antimicrobiële middelen op het voorschrijfgedrag in veertien Vlaamse praktijken voor kleine huisdieren

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ABSTRACT

A prospective study was performed to explore the prescription habits in fourteen first-line, small animal practices during first consultations of cats and dogs. Consultations one month prior to the implementation of antimicrobial use guidelines and at least 20 days thereafter were examined. Differences in the proportion of consultations during which antimicrobials were prescribed, were assessed. Additionally, changes in the choice of active substance were critically evaluated against the introduced antimicrobial use guidelines. The proportion of consultations where antimicrobials were prescribed decreased in cats and dogs (both –12%) after the introduction of the antimicrobial use guidelines. There was an increase of consultations of cats (+13%) and dogs (+10%) where veterinarians handled according to those guidelines. However, an increase in the prescription of third-choice antimicrobials and highest priority critically important antimicrobials was noticed both in cats (+8% and +12%, respectively) and dogs (both +5%). This unexpected increase invites to create extra awareness amongst prescribers.

SAMENVATTING

Aan de hand van een prospectieve studie werd het voorschrijfgedrag met betrekking tot antimicrobiële middelen onderzocht in veertien eerstelijnspraktijken voor kleine huisdieren. Verschillen in het aantal consultaties van katten en honden waarbij antimicrobiële middelen werden voorgeschreven, werden onderzocht gedurende één maand voor en minstens twintig dagen na het invoeren van de adviezen betreffende het gebruik van antimicrobiële middelen. Daarnaast werden ook veranderingen in de keuze van actieve substanties vergeleken met de adviezen. Het aantal consultaties waarbij antimicrobiële middelen werden voorgeschreven daalde zowel bij honden als katten (−12% bij beide diersoorten) na het invoeren van de adviezen. Er was een stijging in het aantal consultaties bij katten (+13%) en honden (+10%) in de praktijken waar de dierenartsen handelden volgens de adviezen. Er werd echter ook een stijging vastgesteld in het voorschrijven van derdekeuze- en kritisch belangrijke antimicrobiële middelen bij kat (+8% en +12%, respectievelijk) en hond (beide +5%). Deze onverwachte stijging wijst erop dat het verantwoord gebruik van antimicrobiële middelen verder onder de aandacht dient te worden gebracht.

INTRODUCTION

The use of antimicrobials in veterinary medicine may promote the selection of bacteria with acquired resistance genes in animals and humans (Dewulf et al., 2007; Magalhães et al., 2010; Burow et al., 2014; Chantziaras et al., 2014). For long, research has focused on food animals as reservoir for antimicrobial resistance (Bates et al., 1994; Robredo et al., 2000; Wooldridge, 2012), but also companion animals play

a role (Harvey et al., 1994; Guardabassi et al., 2004; Loeffler et al., 2005; Lloyd, 2007; Bramble et al., 2011). It is likely that intense contact with companion animals is a risk factor for the transfer of antimicrobial resistance between humans and animals (Guardabassi et al., 2004; Weese and van Duijkeren, 2010).

There is plenty of evidence that decreased antimicrobial use in veterinary medicine is beneficial in reducing the occurrence of antimicrobial resistance and is not necessarily associated with inferior production results. For example, the prevalence of vancomycin-resistant enterococci in food animals (Bager et al., 1999; Klare et al., 1999; Pantosti et al., 1999; Van den Bogaard et al., 2000) and humans (Klare et al., 1999) decreased after the ban of avoparcin in animal feed. In 2015, the prevalence of extended spectrum beta-lactamases/AmpC producing *E. coli* in poultry in the Netherlands decreased in comparison with previous years, possibly because of a reduced antimicrobial use (Anonymous, 2016). No negative effects in swine productivity were noticed after a substantial decrease in antimicrobial consumption (Aarestrup et al., 2010; Postma et al., 2016).

Penicillins and cephalosporins are by far the most used antimicrobial drugs in dogs and cats (Watson and Maddison, 2001; Rantala et al., 2004; Regula et al., 2009; Thomson et al., 2009; Escher et al., 2011; Mateus et al., 2011; Murphy et al., 2012; Pleydell et al., 2012). For Belgium, this was confirmed by the sixth European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) report, which described that in 2014, mainly penicillins and first- and second-generation cephalosporins were used in small animals (EMA, 2016).

In human medicine, antimicrobial use guidelines have been developed and implemented as one of the strategic goals to optimize antimicrobial use (Goldmann et al., 1996), and they have shown to have a positive effect on the antimicrobial prescribing behavior (Smith et al., 2012; Lee et al., 2016). In veterinary medicine, only in a limited number of studies, the impact of antimicrobial guidelines has been investigated. In Germany, antimicrobial guidelines seems to decrease antimicrobial drug use in pigs (Ungemach et al., 2006). In a Canadian veterinary teaching hospital, the implementation of antimicrobial use guidelines resulted in a decrease in the number of prescriptions and the use of first-generation cephalosporins, fluoroquinolones, penicillins, tetracyclines and third-line drugs (carbapenems and vancomycin) (Weese, 2006). Although it was not the main focus of their study, some researchers have looked into the role of guidelines for antimicrobial use in small animals (Rantala et al., 2004; Thomson et al., 2009; Escher et al., 2011; Pleydell et al., 2012). They concluded that the compliance was moderate to good, but varied with the disease, and that the level of performing the necessary diagnostic steps could be improved.

In 2014, the Belgian centre of expertise on Antimicrobial Consumption and Resistance in Animals (AMCRA) provided antimicrobial guidelines for the practising veterinarians. Some antimicrobial drugs are classified as critically important and highest priority critically important antimicrobials based on the lists provided by the World Health Organisation (WHO) and the World Organisation for Animal Health (OIE) (WHO, 2011; OIE, 2015). The aim of this study was to evaluate the impact of introducing these antimicrobial use guidelines on the prescription habits of veterinarians in small animal practices in Flanders. The hypotheses were that veterinarians would refrain from the prescription of antimicrobials, if not needed according to the guidelines, and that they would prescribe less critically important antimicrobials.

MATERIALS AND METHODS

Study design

The antimicrobial use guidelines include twenty-five clinical conditions and describe what requirements should be met for diagnosis and whether antimicrobial treatment is advised (Table 1). If antimicrobials are indicated, the guidelines list first-, second- and/or third-choice antimicrobials per condition. This classification is based on the scientific literature regarding antimicrobial susceptibility, pharmacokinetics, pharmacodynamics and clinical efficacy of the therapy for a given indication. The guidelines are available in Dutch and French at www.e-formularium.be.

For practical reasons, the veterinarians were selected in the regions of Antwerp and East-Flanders by using a website that listed all veterinarians per geographic region (VetWorks, 2011). The selected veterinarians received an email with an invitation to participate in the study, and were subsequently contacted by phone to make an appointment if they had indicated that they were willing to participate.

One of the authors paid personal visits to all participating practices. During the first visit, the consultations from at least a month prior to the visit were extracted from the practice management system. This information per consultation contained the animal species (cat/dog), condition and/or symptoms and antimicrobial treatments. Next, the antimicrobial use guidelines were explained and distributed as a pocket-size booklet (AMCRA, 2014). The veterinarians were asked to implement the guidelines as much as possible when treating cats and dogs during at least twenty working days. They were also instructed to write down in a logbook when and why they had chosen not to follow the guidelines. During the second practice visit, the consultations between the first and second practice visits were extracted from the management system. The veterinarians filled in a questionnaire regarding perceived usefulness of the antimicrobial use guidelines (e.g. user-friendliness, feasibility, structure of the book) using a five-point Likert scale (1 = totally

disagree, 2 = disagree, 3 = neutral, 4 = agree and 5 = totally agree) and the logbook information was collected.

Definitions

The clinical conditions included in the study were listed in six groups: skin, respiratory tract, digestive tract, urogenital tract, ear and other (osteomyelitis, sepsis and antimicrobial prophylaxis) (Table 1). Five types of consultations were not withheld for further evaluation: 1) consultations that concerned other conditions than those described in the antimicrobial use guidelines, 2) when details on the animal species, condition/symptoms and/or active substance were missing, 3) consultations where multiple diagnoses were mentioned, because it was impossible to determine for which condition the antimicrobials were prescribed, 4) consultations that were re-examinations and 5) consultations for chronic pathologies.

Prescribing an off-label product was defined as prescribing an active substance not registered for cats or dogs and/or the condition.

Statistical analysis

The first outcome of interest was whether the veterinarian had prescribed antimicrobials during a consultation, irrespective of whether or not the guidelines were followed. A second outcome of interest was

Table 1. The conditions included in the antimicrobial use guidelines and indication for antimicrobial use according to these guidelines.

Condition	Antimicrobials indicated?
Pyoderma	
• Surface	No
• Superficial	Yes
• Deep	Yes
Wounds and skin abscess formation	
• Non-complicated	No
• Signs of systemic illness, infected wound, extension of the abscess to deeper tissues	Yes
Rhinitis/sinusitis dog	No
Laryngitis/tracheitis dog	
• Non-complicated	No
• Signs of systemic illness, deeper infections	Yes
Bronchitis/(broncho)pneumonia/canine infectious tracheobronchitis	
• Non-complicated	No
• Signs of systemic illness, deeper infections	Yes
Bronchitis/(broncho)pneumonia cat	
• Non-complicated	No
• Signs of systemic illness, deeper infections	Yes
Upper respiratory tract disease cat	
• Conjunctivitis	No
• Rhinitis	No
• Upper respiratory tract disease	Yes
Infections of the mouth cavity	No, unless abscess formation and fistulation are confirmed or in case of a secondary bacterial infection
Infections of the gums and parodontium	No, unless the animal is immunocompromised
Gastro-enteritis	No, unless the presence of blood in the vomit or stool, fever, leucocytosis/neutropenia, left shift in the blood or indications for an aspiration pneumonia
Hepatitis and/or cholangitis	No, unless of bacterial origin
Abscesses of the anal gland	No, unless abscess formation and/or fistulation
Lower urinary tract infection dog	Yes
Lower urinary tract infection cat	No, unless culture and sensitivity test confirm a bacterial origin
Pyelonefritis	Yes
Prostatitis dog	Yes
Balanoposthitis dog	No, unless culture results confirm a bacterial origin
Orchitis dog	No
Vaginitis	No, unless culture results confirm a bacterial origin
Endometritis/pyometra	Yes
Osteomyelitis	Yes
Otitis externa dog	Yes, topical only
Otitis externa cat	Yes, topical only
Sepsis	Yes
Antimicrobial prophylaxis	
• Clean surgeries	No
• Clean-contaminated, contaminated or dirty surgeries	Yes

* Signs of systemic illness such as anorexia, and fever.

Table 2. The number of consultations included in the analysis and the number of consultations where antimicrobials were prescribed before and after the introduction of the antimicrobial use guidelines per veterinary practice and animal species.

Veterinary practice	Cat					Dog				
	Consults prescribed / Total consults					Consults prescribed / Total consults				
Veterinary practice	Before guidelines		After guidelines		Evolution	Before guidelines		After guidelines		Evolution
	n	%	n	%	%	n	%	n	%	%
1	9/10	90%	7/11	64%	-26%	13/15	87%	11/12	92%	+5%
2	33/33	100%	9/9	100%	+0%	78/78	100%	53/53	100%	+0%
3	24/49	49%	41/94	44%	-5%	54/99	55%	77/166	46%	-8%
4	5/8	63%	13/13	100%	+38%	23/24	96%	25/25	100%	+4%
5	12/13	92%	5/10	50%	-42%	16/17	94%	17/27	63%	-31%
6	5/6	83%	9/9	100%	+17%	20/20	100%	15/15	100%	+0%
7	5/5	100%	1/1	100%	+0%	21/21	100%	20/20	100%	+0%
8	10/16	63%	12/15	80%	+18%	12/17	71%	26/31	84%	+13%
9	9/9	100%	8/9	89%	-11%	7/8	88%	7/11	64%	-24%
10	9/13	69%	3/10	30%	-39%	2/5	40%	5/8	63%	+23%
11	5/8	63%	29/47	62%	-1%	14/19	74%	76/116	66%	-8%
12	8/8	100%	2/2	100%	+0%	6/6	100%	9/9	100%	+0%
13	1/5	20%	3/8	38%	+18%	7/10	70%	7/14	50%	-20%
14	7/15	47%	6/9	67%	+20%	4/8	50%	8/15	53%	3%
Total	**142/198**	**72%**	**148/247**	**(60%)**	**-12%**	**277/347**	**80%**	**356/522**	**68%**	**-12%**

whether the veterinarian had handled according to the antimicrobial use guidelines during a consultation. Whenever antimicrobials were prescribed, the following two requirements had to be fulfilled to be in agreement with the antimicrobial use guidelines: 1) a clear indication to use antimicrobials as part of the treatment protocol was present and 2) a first- (or second-) choice antimicrobial was selected. For each outcome of interest, a generalized linear mixed model was fitted to examine the association with the introduction of the antimicrobial guidelines, the clinical condition and the self-reported frequency of working according to the guidelines (i.e. whether they indicated to work 'sometimes' or 'most of the times' according to the guidelines. A logit link function and binomial distribution were assumed. To account for clustering of consultations in veterinary practices, a random effect for veterinary practice was included. Separate models were made for cats and dogs.

To examine the strength of the association between the prescription of antimicrobials and whether antimicrobials were indicated according to the guidelines, odds ratios (OR) and Cohen's kappa coefficients were estimated with 95% confidence intervals (CI) (McHugh, 2012).

The prescription of an active antimicrobial substance was expressed as percentage compared to the total number of antibiotic prescriptions. The same accounts for the numbers of first-, second- and third-choice, off-label and highest priority critically important antimicrobials. The data on active substances, first-, second-, third-choice antimicrobials, off-label, highest priority critically important antimicrobials before and after the introduction of the antimicrobial use guidelines and the self-reported frequency of working according to the guidelines were analyzed using a Pearson χ^2 test. When the number of consultations was lower than five, the Fisher's exact test was applied. All analyses were performed with SPSS 22.0 (IBM Corp, Armonk, NY) or SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The significance level was set at 5%.

RESULTS

Of the 62 invited veterinarians, 23 expressed their willingness to participate. Six veterinarians discontinued their participation after the first visit to the practice, because they feared that it would be too much work to record all data or to consult the antimicrobial use guidelines. One veterinarian was not convinced about the purpose of the study, because in his opinion, only a limited amount of antimicrobials are generally being prescribed in small animal practices. One veterinarian only handed over data on consultations from the period before the introduction of the antimicrobial use guidelines, and one veterinarian only handed in data on consultations where antimicrobials had been prescribed. As a result, complete datasets for analysis containing 1314 consultations were available from 14 veterinary practices.

Table 3. Overview of the number of consultations, during which antimicrobials were prescribed and whether the guidelines were followed per animal species and clinical condition, before and after the introduction of the antimicrobial guidelines.

Cat										
	Consults prescribed / Total consults					Consults according to guidelines / Total consults				
Clinical conditions	Before guidelines		After guidelines		Evolution	Before guidelines		After guidelines		Evolution
	n	%	n	%		n	%	n	%	
Skin	67/69	97%	43/47	91%	-6%	5/69	7%	5/47	11%	+4%
Respiratory	17/20	85%	27/33	82%	-3%	4/20	20%	7/33	21%	+1%
Digestive	17/30	57%	33/54	61%	+4%	12/30	40%	23/54	43%	+3%
Urogenital	17/23	74%	25/33	76%	+2%	5/23	22%	10/33	30%	+8%
Ears	3/4	75%	6/7	86%	+11%	1/4	25%	2/7	29%	+4%
Other	21/52	40%	14/73	19%	-21%	32/52	62%	59/73	81%	+19%
Dog										
	Consults prescribed / Total consults					Consults according to guidelines / Total consults				
Clinical conditions	Before guidelines		After guidelines		Evolution	Before guidelines		After guidelines		Evolution
	n	%	n	%		n	%	n	%	
Skin	77/82	94%	68/83	82%	-12%	21/82	26%	26/83	31%	+5%
Respiratory	25/29	86%	32/35	91%	+5%	8/29	28%	7/35	20%	-8%
Digestive	57/83	69%	107/166	64%	-4%	35/83	42%	76/166	46%	+4%
Urogenital	18/21	86%	26/31	84%	-2%	9/21	43%	16/31	52%	+9%
Ears	33/39	85%	46/54	85%	+0%	20/39	51%	32/54	59%	+6%
Other	67/93	72%	77/153	50%	-22%	26/90	29%	75/151	50%	+21%

Cats

The database contained 198 cat consultations before and 247 consultations after the introduction of antimicrobial use guidelines. Antimicrobials were prescribed in 72% (142/198) and in 60% (148/247) of the consultations before and after the introduction of antimicrobial use guidelines, respectively (Table 2). Although a decrease was noticed, the introduction of antimicrobial use guidelines did not significantly influence the proportion of consultations where antimicrobials are prescribed ($P = 0.71$). The prescription pattern varied significantly with the clinical condition ($P < 0.001$) (Table 3). As expected, antimicrobials were more likely prescribed when indicated by the guidelines, 92% (36/39) versus 67% (106/159) before (OR 6.0, 95% CI 1.8; 20.4) and 88% (38/43) versus 54% (110/204) after (OR 6.5, 95% CI 2.5; 17.2) the introduction of the guidelines (Table 4). However, it was also noticed that a very substantial amount of prescriptions were actually not indicated, 54% (106/198) before and 45% (110/247) after the introduction of the guidelines, respectively. The corresponding values for Cohen's kappa (0.13 and 0.17 before and after, respectively) indicate only a slight agreement between the prescription and the actual indication of antimicrobials (McHugh, 2012). The percentage of consultations handling according to the antimicrobial use guidelines increased from 30% to 43% after the introduction of antimicrobial use guidelines ($P = 0.24$). Handling according to the guidelines varied significantly with the clinical condition ($P < 0.001$) (Table 3).

Amoxicillin clavulanate and third-generation cefovecin (critically important) were the most commonly prescribed antimicrobials before and after the introduction of the antimicrobial use guidelines although their relative proportions changed (Table 5). There was a significant difference in the relative frequency of prescriptions before and after the guidelines for the following antimicrobials: amoxicillin clavulanate (-15%, $P < 0.001$), cefovecin (+11%, $P < 0.01$) and doxycycline (+6%, $P < 0.01$). An overall significant shift in the prescription pattern concerning first-, second- and third-choice antimicrobial as well as off-label products was noticed after the introduction of antimicrobial use guidelines ($P = 0.02$). The relative number of prescriptions of second-choice antimicrobials decreased by 16%, while the prescription of first-choice (+4%), third-choice (+8%) antimicrobials and off-label products (+3%) increased. Furthermore, the relative number of prescriptions of highest priority critically important antimicrobials increased by 12% ($P = 0.02$) after the introduction of the antimicrobial use guidelines.

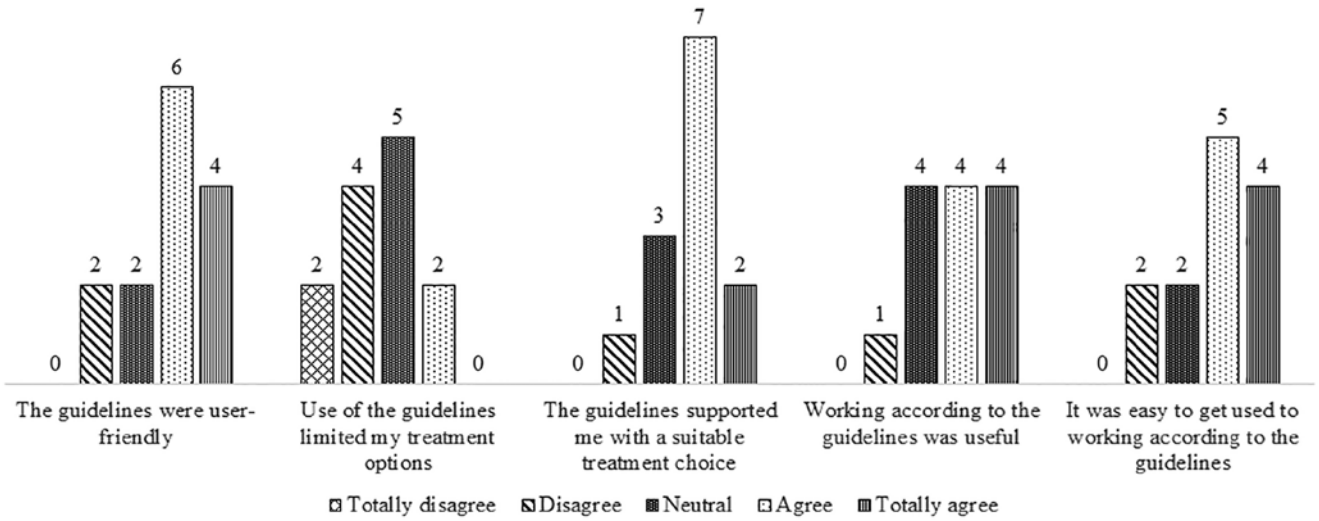

Figure 1. Results of the questionnaire regarding perceived usefulness of the antimicrobial guidelines. A five-point Likert-scale was used: (1) strongly disagree, (2) disagree, (3) undecided, (4) agree and (5) strongly agree. Thirteen veterinarians answered all questions, one veterinarian only answered whether the guidelines were user-friendly.

Dogs

Antimicrobials were prescribed in 80% (277/347) before and in 68% (356/522) of the consultations after the introduction of antimicrobial use guidelines (Table 2). Similarly as for cats, this decrease was not significantly associated with the introduction of antimicrobial use guidelines (P = 0.49). The prescription pattern varied significantly with the clinical condition (P < 0.001) (Table 3). Antimicrobials are more likely prescribed when indicated by the guidelines, 88% (107/122) versus 76% (170/225) before (OR 2.3, 95% CI 1.2; 4.3) and 87% (138/159) versus 60% (218/363) after (OR 4.4, 95% CI 2.6; 7.2) the introduction of the guidelines (Table 4). Again, a considerable amount of prescriptions were actually not indicated, 49% (170/347) before and 42% (218/522) after the introduction of the guidelines, respectively. The corresponding values for Cohen's kappa (0.09 and 0.20 before and after, respectively) indicate only a slight agreement between the prescription and the actual indication of antimicrobials. The percentage of consultations handling according to the antimicrobial use guidelines increased from 35% to 45%. This increasing trend was not significantly associated with the introduction of antimicrobial use guidelines (P = 0.13), but varied significantly with the clinical condition (P < 0.001) (Table 3).

Amoxicillin clavulanate and cephalexin were the most commonly prescribed antimicrobials before and after the introduction of the guidelines. There was a significant difference in the relative frequency of pre-

Table 4. Associations between the prescription of antimicrobials and whether the prescription was indicated according to the guidelines both before and after the introduction of the guidelines per animal species.

	Cat				Dog			
Before the guidelines	n=198		Indicated		n=347		Indicated	
			Yes	No			Yes	No
	Prescribed	Yes	36	106	Prescribed	Yes	107	170
		No	3	53		No	15	55
	OR:	6.0	95% CI: 1.8; 20.4		OR:	2.3	95% CI: 1.2; 4.3	
	Kappa:	0.13	95% CI: 0.06; 0.20		Kappa:	0.09	95% CI: 0.03; 0.16	
After the guidelines	n=247		Indicated		n= 522		Indicated	
			Yes	No			Yes	No
	Prescribed	Yes	38	110	Prescribed	Yes	138	218
		No	5	94		No	21	145
	OR:	6.5	95% CI: 2.5; 17.2		OR:	4.4	95% CI: 2.6; 7.2	
	Kappa:	0.17	95% CI: 0.10; 0.25		Kappa:	0.20	95% CI: 0.14; 0.26	

OR = odds ratio; CI = confidence interval

Table 5. Active substances prescribed in cats and dogs, before and after the introduction of antimicrobial use guidelines.

Antimicrobial	% Cats (n)			% Dogs (n)		
	Before	After	D	Before	After	D
Amoxicillin	15% (27)	10% (17)	-5%	5% (16)	9% (39)	+4%*
Amoxicillin clavulanate	29% (52)	14% (23)	-15%*	25% (86)	20% (84)	-5%*
Azithromycin		1% (1)	+1%			
Cephalexin	3% (5)	8% (13)	+5%	21% (89)	22% (94)	+1%
Cephalosporins				0.3% (1)		
Cefazolin	3% (5)	2% (4)	-1%	13% (44)	9% (37)	-4%*
Cefoperazone					0.2% (1)	
Cefovecin	31% (55)	42% (70)	+11%*	3% (9)	1% (6)	-2%
Chloramphenicol	1% (2)	1% (1)				
Clindamycin	2% (4)	2% (3)		1% (3)	4% (16)	+3%*
Difloxacin	3% (5)	1% (1)	-2%		0.2% (1)	
Doxycycline		6% (10)	+6%*	3% (10)	2% (9)	-1%
Enrofloxacin	3% (5)	4% (7)	+1%	3% (12)	7% (30)	+4%*
Fusidic acid/ Framycetin		1% (1)	+1%	2% (6)	2% (9)	
Gentamicin	1% (1)	1% (2)		3% (12)	4% (15)	
Lincomycin	1% (1)		-1%		1% (3)	+1%
Lincomycin Spectinomycin	2% (3)	1% (1)	-1%			
Marbofloxacin	1% (1)	2% (3)	+1%	5% (15)	4% (15)	-1%
Metronidazole				4% (10)	4% (16)	
Metronidazole Spiramycin	3% (5)	4% (6)	+1%	5% (17)	5% (22)	
Mupirocin				0.3% (1)		
Neomycin		1% (1)	+1%	2% (8)	1% (5)	-1%
Ofloxacin	1% (2)	1% (2)			1% (2)	
Orbifloxacin					2% (8)	+2%*
Oxytetracyclin /Polymyxin B	1% (1)	1% (1)		1% (3)	0.2% (1)	
Polymyxin B	1% (1)		-1%	1% (4)	2% (8)	+1%
Pradofloxacin	1% (1)		-1%	0.3% (1)		
Procaine Benzyl penicillin		1% (1)	+1%			
Procaine Benzyl penicillin/ Neomycin	1% (1)		-1%		1% (2)	+1%
Procaine Benzyl penicillin/ Streptomycin Nafcillin	1% (1)		-1%			
Tobramycin	1% (1)		-1%			
Trimethoprim Sulphonamides	1% (1)		-1%	1% (2)	1% (2)	
Total number of prescriptions	**180**	**168**		**349**	**425**	

D = difference between the percentage before and after the introduction of antimicrobial use guidelines. * Significant difference (5% significance level following χ^2 test or Fisher's exact test).

scriptions before and after the guidelines for the following antimicrobials: amoxicillin (+4%, P = 0.02), amoxicillin clavulanate (−5%, P = 0.03), cefazolin (−4%, P = 0.04), clindamycin (+3%, P = 0.01), enrofloxacin (+4%, P = 0.04) and orbifloxacin (+2%, P = 0.01) (Table 5). An overall significant shift in the prescription pattern concerning first-, second- and third-choice antimicrobial as well as off-label products was noticed after the introduction of antimicrobial use guidelines (P = 0.04). The relative number of prescriptions of off-label antimicrobials decreased by 10%, while the prescription of first- (+5%), second- (+1%) and third- (+5%) choice antimicrobials increased. Furthermore, the relative number of prescriptions of highest priority critically important antimicrobials increased by 5% (P = 0.06) after the introduction of the antimicrobial use guidelines.

Logbooks and questionnaires

Unfortunately, the logbooks were not consistently used to write down the reasons for divergence from antimicrobial use guidelines. Therefore, these data could not be used for further evaluation. Thirteen veterinarians answered all questions, while one veterinarian only answered whether the guidelines were user-friendly (Figure 1). The Likert-scale questions revealed that veterinarians were positive about the antimicrobial use guidelines: the guidelines were evaluated as user-friendly (mean score 3.9) and useful (3.8). The guidelines supported the veterinarians with a suitable treatment choice (3.8) and did not limit the treatment options of the veterinarians (2.5). The veterinarians also indicated that it was easy to get used to work according to the guidelines (3.8). Veterinarians

mentioned the format and size of the booklet with antimicrobial use guidelines and the clear grouping of the different conditions as positive features.

Nine veterinarians indicated that they were most of the time able to work according to the guidelines; five sometimes. Surprisingly, antimicrobials were more likely prescribed during consultations of veterinarians who stated to work most of the time according to the guidelines compared to veterinarians who stated to work sometimes according to the guidelines, both in cats (OR = 9.2; 95% CI 2.9; 29.6) and dogs (OR = 19.2; 95% CI = 2.9; 124.8). Furthermore, the guidelines were less likely to be followed during consultations of the 'most of the time' veterinarians compared to the 'sometimes' veterinarians, both in cats (OR = 0.26; 95% CI 0.12; 0.57) and dogs (OR = 0.47; 95% CI 0.23; 0.98). The most frequently mentioned reason to diverge from the guidelines was the use of cefovecin in cats because of the perceived difficulty to administer tablets in contrast to the user-friendly injection. Other reasons to diverge from the guidelines were practical reasons (drug not in stock), good experience with other antimicrobials for that specific condition, and declination of additional diagnostic tests by the owner, preventing to comply with the diagnostic requirements before prescribing an antimicrobial.

DISCUSSION

After the introduction of the antimicrobial use guidelines, a decrease was observed in the percentage of consultations where antimicrobials were prescribed and more prescriptions were made according to the guidelines. Unfortunately, the observed improvements in the number of consultations without use of antimicrobials and according to the guidelines were not statistically significant when taking into account the clustering of the results within the veterinary practices. This is likely the result of the fact that the effect of the introduction of the guidelines was not consistent for the participating veterinarians, in combination with the limited sample size (14 veterinary practices) (Table 2). The increase in prescription of third-choice antimicrobials after the introduction of antimicrobial use guidelines was also an unexpected result. Nevertheless, the results of this study gave some food for thought concerning the prescription habits in small animal practices and antimicrobial use guidelines.

In this study, mainly penicillins and third- and fourth-generation cephalosporins were prescribed in cats, whereas penicillins and first- and second-generation cephalosporins were predominant in dogs. These results are comparable to previous data obtained in Belgium and the rest of Europe (Regula et al., 2009; Mateus et al., 2011; De Briyne et al., 2014) and also aligned to a certain extent with the results described in a Canadian study, conducted in 2004, in which the percentage of cases where antimicrobials were prescribed, decreased (Weese, 2006). Unlike the Canadian study, where the prescription of first-, second- as well as third-choice antimicrobials decreased, in the present study, the prescription of second-choice antimicrobials decreased significantly in cats, while the prescription of the third-choice antimicrobials increased after the introduction of antimicrobial use guidelines. However, the study designs differed considerably. In the previous study, six years before and four years after the introduction of antimicrobial use guidelines were assessed. Furthermore, it did not distinguish between cats and dogs, and the data were gathered in a small animal teaching hospital, not in individual small animal practices. It is likely that better results were obtained because of the fact that veterinarians working in a tertiary care veterinary teaching hospital might be more strict in respecting the guidelines, and it might be easier to implement guidelines in one referral practice than in multiple, first-line practices. Moreover, the longer study period in the Canadian study made it possible for the veterinarians to get familiar with these guidelines and to implement them in their routine. In the present study, the veterinarians were only briefly personally instructed on how to work with the antimicrobial use guidelines, and the study period may have been too short.

Notable was the unexpected increase of the prescription of third-choice antimicrobials in cats and dogs; in particular, the prescription of cefovecin increased substantially. Cefovecin is a broad-spectrum, third-generation cephalosporin registered for the treatment of cats and dogs and is classified among the highest priority critically important antibiotics (WHO, 2011). In cats, the antimicrobial activity following a single injection lasts up to 14 days (SPC Convenia, 2013). The parenteral administration route makes cefovecin an easy antimicrobial to administer. Previous studies have indicated that the ease of administration is the key factor explaining the popularity of this antimicrobial (De Briyne et al., 2013), as also stated by the participating veterinarians. The preference is likely also influenced by anticipated low owner compliance in administering a short-term oral antimicrobial therapy (Grave and Tanem, 1999).

The results from this study indicate that antimicrobials were prescribed far more often than indicated both before and after the implementation of the antimicrobial use guidelines, showing that there is still a large margin for the reduction of antibiotic use in small animal medicine. It has been described that small animal veterinarians mainly base their decision whether or not to prescribe antimicrobials on the observed clinical signs. Less frequently, they await the results of bacteriological culture or cytology (Hughes et al., 2012). Other factors that are taken into account are the ease of administration, financial constraints and client expectations (Hughes et al., 2012). Moreover, the veterinarians' self-reported frequency of working according to the antimicrobial use guidelines did not correspond with the measured frequencies of prescribing antimicrobials and working according to the

guidelines. This non-correspondence together with the large margin for the reduction of antimicrobial use might suggest that veterinarians are not always aware of their actual antimicrobial use and that increasing the self-consciousness in combination with measuring objectively the antimicrobial use is needed. For instance, a centralized data-collection system on antimicrobial use in small animals, existing already in several European countries for farm animals, can be useful to measure the antimicrobial use in a standardized manner, to compare the use between veterinary practices and to provide feedback to veterinary practices about their use. Antimicrobial use guidelines can, in combination with this monitoring, be a useful tool to support veterinarians towards a more responsible use of antimicrobials.

The results of this study suggest that antimicrobial use guidelines can be a supportive tool for a more responsible use of antimicrobials, which can serve as basis for further research. With a larger sample size, a more balanced design and a longer observation period, more detailed, condition-specific and clear results on the usefulness of antimicrobial guidelines may be obtained. Another step is to develop communication strategies to inform large groups of veterinarians about the need of the responsible use of antibiotics and about the benefits of using the antimicrobial use guidelines. Together with the aforementioned monitoring of antimicrobial use, all of this may help in targeted communication with and training of small animal veterinarians, aiming at a restricted and more responsible use of antibiotics.

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Pyothorax in cats and dogs

Pyothorax bij de kat en de hond

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ABSTRACT

Pyothorax, or thoracic empyema, is an infection of the pleural space, characterized by the accumulation of purulent exudate. It is a life-threatening emergency in dogs as well as in cats, with a guarded prognosis. Dyspnea and/or tachypnea, anorexia and lethargy are the most typical clinical signs. Diagnosis is usually straightforward, based on the clinical symptoms combined with pleural fluid analysis, including cytology and bacterial culture. Most commonly, oropharyngeal flora is isolated in the pleural fluid. Treatment can be medical or surgical, but needs to be immediate and aggressive. In this article, an overview of the various causes of both feline and canine pyothorax with its similarities and differences is provided. Epidemiology, symptoms, diagnosis, treatment and prognosis are discussed.

SAMENVATTING

Pyothorax, of thoraxempyeem, is een infectie van de pleurale holte, gekenmerkt door een accumulatie van purulent exudaat. Het is een levensbedreigende aandoening, zowel bij honden als bij katten, met een gereserveerde prognose. Dyspnee en/of tachypnee, anorexie en lethargie zijn de meest voorkomende symptomen. De diagnose is meestal gemakkelijk te stellen aan de hand van de klinische symptomen en onderzoek van het pleurale vocht, inclusief een cytologisch en bacteriologisch onderzoek. Meestal wordt orofaryngeale flora geïsoleerd in de pleurale effusie. De behandeling kan zowel medicamenteus als chirurgisch zijn, maar moet snel en agressief ingesteld worden. In dit artikel wordt een overzicht gegeven van de meest voorkomende oorzaken van zowel feliene als caniene pyothorax, waarbij gelijkenissen en verschillen worden besproken. Epidemiologie, klinische symptomen, diagnose, behandeling en prognose komen uitgebreid aan bod.

INTRODUCTION

In dogs as well as in cats, pyothorax can be a life-threatening disease. It is defined as the presence of septic exudate in the pleural space (Ettinger and Feldman, 2010). Patients with pyothorax are usually presented with dyspnea and/or tachypnea and it might be difficult to determine the underlying cause straight away (Murphy and Papasouliotis, 2011a; Firth and Boag, 2012; Epstein, 2014). In general, the first step in dealing with dyspneic patients consists of providing a stress-free environment with sufficient oxygenation to maximize the breathing comfort of the animal. The clinical examination may have to be postponed, but a close inspection of the breathing pattern will help in the initial localization of the problem (Beatty and Barrs, 2010; Murphy and Papasouliotis, 2011a; Sigrist et al., 2011; Firth and Boag, 2012). On auscultation, pleural space disease is characterized by muffled heart and lung sounds. In case of pleural effusion, auscultation will be muffled ventrally, often in combination with increased lung sounds dorsally. The presence of air (pneumothorax) decreases dorsal lung sounds (Murphy and Papasouliotis, 2011a; Firth and Boag, 2012; Epstein, 2014). After initial stabilization, appropriate measures should be taken to relieve the discomfort of the animal. In many cases, immediate thoracocentesis is necessary (Beatty and Barrs, 2010; Firth and Boag, 2012). This may be therapeutic as well as diagnostic, because pleural fluid provides im-

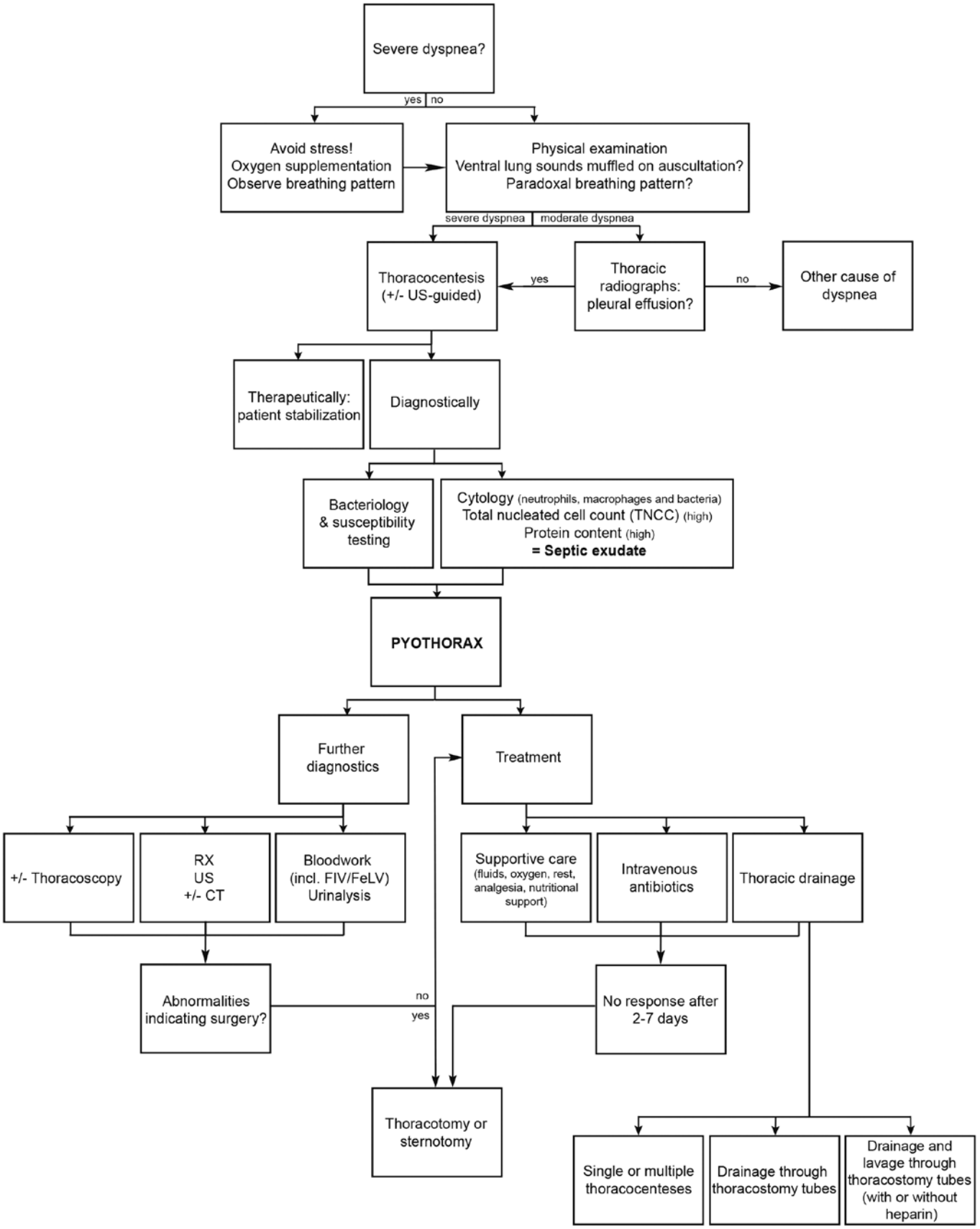

Figure 1. Flow chart for diagnostic and therapeutic management of pyothorax in dogs and cats.

portant information concerning the underlying cause (Murphy and Papasouliotis, 2011a; Epstein, 2014).

Although the diagnosis of pleural space disease and pleural effusion is usually straightforward, long-term treatment may be more challenging and depends largely on the nature of the effusion (Murphy and Papasouliotis, 2011a; Epstein, 2014). In case of pyothorax, pleural effusion is characterized by a septic exudate and medical management should at least consist of supportive care, systemic antibiotic therapy and drainage of the pleural fluid, which can be achieved through single or multiple thoracocenteses or through placement of thoracic drains, with or without thoracic lavage. Surgical management through thoracic surgery or thoracoscopy may be necessary in selected cases (Rooney and Monnet, 2002; Swinbourne et al., 2011). In this review, the general approach of pyothorax in clinical practice is focussed upon (Figure 1). The basic epidemiology and pathophysiology are briefly explained to better understand clinical signs and diagnostic measures, and different treatment options are discussed to aid the clinician in making the best therapeutic choices for each patient individually.

EPIDEMIOLOGY

Pyothorax is most frequently seen in middle-aged dogs and cats with a mean age of three to six years (Demetriou et al., 2002; Waddel et al., 2002; Barrs et al., 2005; Boothe et al., 2010). In some studies, outdoor male dogs and cats were more frequently affected, probably because young, male animals have a greater likelihood to roam and fight, and therefore obtain penetrating injuries more easily (Demetriou et al., 2002; Waddel et al., 2002; Malik et al., 2006; Boothe et al., 2010). There is no clear data available concerning the actual incidence of pyothorax in dogs or in cats. Potential underlying causes include penetrating trauma or bite wounds, migrating foreign bodies, parasites, neoplasia, hematogenous spread of extra-thoracic or intra-thoracic infections and iatrogenic causes, e.g. thoracic surgery, thoracocentesis (Demetriou et al., 2002; Waddel et al., 2002; Barrs et al., 2005; Klainbart et al., 2005; Malik et al., 2006; Doyle et al., 2009; Boothe et al., 2010; Ettinger and Feldman, 2010).

In dogs, pyothorax is not frequently encountered (Piek and Robben, 2000; Rooney and Monnet, 2002; Johnson and Martin, 2007). The cause of pyothorax is only found in 2 to 19% of canine cases. The most common cause is the migration of foreign bodies. In 40% of dogs with pyothorax that were managed surgically, a foreign body, e.g. grass awn, was the underlying cause (Demetriou et al., 2002). The incidence and type of foreign bodies vary depending on the local geographical flora (Piek and Robben, 2000). English Springer spaniels, Border collies, Labrador retrievers and their crosses are overrepresented due to their large airways, scenting habits, outdoor nature and thus frequent exposure to plant material (Demetriou et al., 2002; Johnson and Martin, 2007; Doyle et al., 2009).

Pyothorax is more frequently seen in cats than in dogs (Waddell et al., 2002; Barrs et al., 2005). A definitive cause is found in 35 to 67% of feline cases. The most common route of infection is thought to be through penetrating bite wounds and abscesses that rupture towards the thoracic cavity, causing bacterial contamination and ultimately pyothorax. Data supporting this hypothesis include a history of wounds in 14 to 40% of cases (Jonas, 1983; Waddell et al., 2002), a seasonal association with more cases in late summer and fall due to increases in fighting behavior (Waddel et al., 2002; MacPhail, 2007) and the isolation of similar bacteria in pyothorax as in bite wound abscesses (Waddell et al., 2002). Increased neutering, confinement and routine treatment with antibiotics after a catfight seem to reduce the incidence of pyothorax (Barrs and Beatty, 2009a). Cats affected by pyothorax are predominantly young outdoor cats from multi-cat households, probably because there is more inter-cat aggression (Waddel et al., 2002). However, these cats also have a greater exposure to upper respiratory tract infections, which is a predisposing event in up to 26% of the feline pyothorax cases. In more recent studies, it has been suggested that aspiration of oropharyngeal flora with parapneumonic spread might be a more frequent cause of pyothorax than bite wounds (Waddell et al., 2002; Barrs et al., 2005; Barrs and Beatty, 2009a).

PATHOGENESIS AND CLINICAL SIGNS

The pleural space and pathophysiology of pleural effusion

The pleural space is a potential space, lined by the visceral and parietal pleura. These serous membranes cover the outer surface of the lungs and inner surface of the thoracic cavity, dividing the pleural space into a left and a right hemithorax, separated by the mediastinum. A thin layer of glycoprotein-rich fluid separates the pleura and allows the different intrathoracic structures to slide freely during respiration (Ettinger and Feldman, 2010). The pleural space of normal cats and dogs contains 0.1 and 0.3 mL/kg of fluid respectively (Epstein, 2014). The production and absorption of this fluid represent a continuous process controlled by Starling's forces. Hydrostatic pressure forces fluid out of the vasculature, while oncotic pressure maintains fluid within the vasculature. Any process that disrupts capillary or interstitial hydrostatic or oncotic pressures, lymphatic drainage or vessel integrity may result in fluid accumulation (Ettinger and Feldman, 2010). The presence of 30 mL/kg of pleural effusion is assumed to cause mild breathing discomfort, while volumes up to 60 mL/kg result in severe dyspnea (Beatty and Barrs, 2010).

Pathogenesis and bacteria associated with feline and canine pyothorax

Bacteria may enter the pleural space through compromised lung parenchyma, bronchi, esophagus or thoracic wall (Light, 2001; MacPhail, 2007). When the pleural space is faced with an infectious organism, it responds with edema and exudation of fluid, proteins and neutrophils into the pleural space. Mesothelial cells then act as phagocytes and trigger an inflammatory response. This results in the release of chemokines, cytokines, oxidants and proteases. The rapidity and extent of progression depend on the type and virulence of the organism, the patient's host defences and the timing and effectiveness of antibiotic treatment. If initial effusion remains untreated, fibropurulent effusions or complex parapneumonic effusions develop. Fibrin is formed in the pleural fluid and results in the formation of adhesions and loculations. A complex parapneumonic effusion progresses to pyothorax when the concentration of leukocytes becomes sufficient to form pus, consisting of fibrin, cellular debris and viable or dead bacteria. If untreated, eventually, an organizing phase occurs with the influx of fibroblasts and the formation of dense fibrous adhesions (Light, 2001; Sevilla et al., 2009; Christie, 2010).

In general, it can be stated that bacteria isolated from canine and feline pyothorax are largely the same and most commonly consist of gram-negative, facultative anaerobic rods and/or obligate anaerobic bacteria, representing oropharyngeal flora (Walker et al., 2000; Demetriou et al., 2002). An important difference between both is the fact that isolated gram-negative, facultative anaerobic rods are predominantly non-enteric in origin in cats, e.g. *Pasteurella* spp., *Pseudomonas* spp., *Actinobacillus* spp., while they are mostly of enteric origin in dogs, e.g. *Escherichia* spp., *Enterobacter* spp., *Klebsiella* spp. (Love et al., 1982; Walker et al., 2000). A common mechanism of infection is the aspiration of oropharyngeal flora and the subsequent colonization of the lower respiratory tract (Piek and Robben, 2000; Barrs et al., 2005; MacPhail, 2007; Barrs and Beatty, 2009a). Oropharyngeal flora may also gain access to the pleural space by aspiration during dental procedures, migrating foreign bodies, e.g. grass awns, penetrating thoracic wounds, e.g. bite wounds, stick injury, hematogenous spread from a distant wound or extension from underlying pulmonary infection (Piek and Robben, 2000; Demetriou et al., 2002; Rooney and Monnet, 2002; Barrs et al., 2005; Doyle et al., 2005; Johnson and Martin, 2007; MacPhail, 2007; Barrs and Beatty, 2009a).

About 20% of feline pyothorax cases are caused by infectious agents other than oropharyngeal flora, including *Rhodococcus equi, Nocardia* spp., *Klebsiella* spp., *Proteus* spp. and *Pseudomonas* spp. (Walker et al., 2000; Demetriou et al., 2002; Barrs and Beatty, 2009a). There is no clear data available concerning the prevalence of pyothorax caused by non-oropharyngeal flora in dogs.

Further, it should be mentioned that filamentous bacteria, e.g. *Nocardia* spp., *Actinomyces* spp., seem to be isolated from pyothorax more often in dogs than in cats (Walker et al., 2000; Sivacolundhu et al., 2001; Demetriou et al., 2002; Barrs et al., 2005). Isolation of *Nocardia* spp. has been reported in 12.5% of feline cases, while it was found in 19% of canine cases (Demetriou et al., 2002). *Actinomyces* spp. are identified in the pleural fluid of 10 to 15% of cats with pyothorax but are present in up to 49% of dogs with pyothorax, although *Actinomyces* spp. form part of the normal oropharyngeal flora in both species. The higher prevalence of *Actinomyces* spp. in canine pyothorax than in feline pyothorax might be explained by its association with grass awn migration in dogs (Sivacolundhu et al., 2001; Rooney and Monnet, 2002; Waddel et al., 2002; Barrs et al., 2005; Doyle et al., 2009).

Clinical signs and findings on physical examination

The duration of clinical signs prior to diagnosis is typically one to two weeks, but it may take months (Barrs and Beatty, 2009a). In dogs, the disease is thought to be chronic at the time of presentation, because of its insidious nature and vague clinical signs (Rooney and Monnet, 2002). Cats are usually presented even later, and by the time clinical signs of respiratory compromise become obvious, minimal respiratory reserve remains (Barrs and Beatty, 2009a).

Both in cats and in dogs, clinical signs include partial or complete anorexia and lethargy or weakness in 80% of cases, followed by dyspnea and/or tachypnea (Demetriou et al., 2002; Mellanby et al., 2002; Rooney and Monnet, 2002; Waddel et al., 2002; Barrs et al., 2005; Doyle et al., 2009; Boothe et al., 2010). The dyspnea of feline patients with pyothorax may be surprisingly subtle and is not noticed by 40% of the owners (Barrs et al., 2005). It is widely accepted that pleural effusion causes a restrictive pattern of respiration, characterized by an increase in respiratory rate and effort (MacPhail, 2007; Murphy and Papasouliotis, 2011a; Sigrist et al., 2011; Firth and Boag, 2012). However, a large study investigating breathing patterns of different causes of dyspnea, revealed that pleural space disease is typically associated with either an asynchronous (inspiration with inward movement of the abdominal wall combined with outward movement of the thoracic wall) or an inverse (inspiration with outward movement of the abdominal wall combined with inward movement of the thorax) breathing pattern (Sigrist et al., 2011). Cats typically adopt a crouched, sternally recumbent posture with abducted elbows and often show open-mouth breathing (Beatty and Barrs, 2010).

Pyrexia and/or exercise intolerance have been reported in almost half of canine cases, while only 28.6% of cats are presented with fever and hardly any

Table 1. Classification of pleural fluid based on total protein (TP) concentration, total nucleated cell count (TNCC) and cytology (Light, 2001; Beatty and Barrs, 2010; Ettinger and Feldman, 2010; Murphy and Papasouliotis, 2011a; Nelson and Couto, 2014; Zoia and Drigo, 2015).

	TP (g/L)	TNCC (/µL)	Cytology	Common causes
Transudate	< 25	< 1 500	Macrophages, mesothelial cells, lymphocytes and non-degenerative neutrophils	Decreased oncotic pressure (e.g. liver disease, protein losing nefropathy, protein losing enteropathy), mildly increased hydrostatic pressure (e.g. right-sided heart failure, pericardial disease)
Modified transudate	25 - 75	1 000 – 7 000	Macrophages, mesothelial cells, lymphocytes and nondegenerative neutrophils	Increased hydrostatic pressure (e.g. right-sided heart failure, pericardial disease), chronic lymphatic obstruction (e.g. neoplasia, diaphragmatic herniation)
Exudate	> 30	> 7 000		Increased vascular permeability
(1) Nonseptic			1. Nondegenerative neutrophils, eosinophils, lymphocytes and macrophages	1. Feline infectious peritonitis (FIP), neoplasia, lung lobe torsion
(2) Septic			2. Degenerative neutrophils, intracellular/extracellular bacteria and macrophages	2. Bacterial pneumonia, penetrating thoracic or esophageal wounds, migrating foreign bodies
Chylous effusion	> 25	< 10 000	Small lymphocytes, nondegenerative neutrophils and macrophages	Leakage from thoracic duct (e.g. neoplasia, idiopathic, congenital, traumatic, pericardial disease, cardiac disease, dirofilariosis, lung lobe torsion)
Hemorrhagic effusion	> 30	< 10 000	Similar to peripheral blood	Hemorrhage (e.g. trauma, coagulopathy, neoplasia, lung lobe torsion)
Neoplastic effusion	> 25	Variable	Inflammatory and reactive mesothelial cells, neutrophils, macrophages and possibly neoplastic cells	Neoplasia of intrathoracical structures (e.g. mediastinal lymfoma, pulmonary carcinoma)

cat shows signs of exercise intolerance (Demetriou et al., 2002; Boothe et al., 2010). Because cats are mostly presented in a very late stage of disease, they are often in a poor body condition (Demetriou et al., 2002; Waddel et al., 2002; Barrs et al., 2005). Coughing has been reported in 14% to 30% of feline cases and up to 15% of cats with pyothorax have concurrent clinical signs of upper respiratory tract infection (oculonasal discharge and/or third eyelid prolapse) (Barrs et al., 2005). Pyothorax is the most common cause of sepsis in cats, and hypothermia, present in 15% of feline cases, should alert for sepsis, particularly when accompanied by bradycardia (Brady et al., 2000; Barrs and Beatty, 2009a). Nevertheless, the absence of bradycardia does not rule out sepsis or pyothorax, since in a study of Barrs et al. (2005), 20% of the cats had tachycardia, whereas bradycardia was not observed. Other clinical presentations occurring in a number of individual cats and dogs are submandibular abscesses, halitosis, cyanosis, lameness and pneumothorax (Demetriou et al., 2002).

On thoracic auscultation, respiratory sounds are decreased to absent. This is more pronounced ventrally and may be asymmetrical (Beatty and Barrs, 2010; Murphy and Papasouliotis, 2011a; Sigrist et al., 2011). Based on auscultation, the initial thoracocentesis should be performed on the side that is most affected (Beatty and Barrs, 2010). Pleural effusion may also create muffled heart sounds (Beatty and Barrs, 2010; Murphy and Papasouliotis, 2011a). The combination of auscultation along with percussion may be helpful in the diagnostic work-up of pleural space disease. On percussion, the presence of free fluid results in a low-pitched resonance (Murphy and Papasouliotis, 2011a).

DIAGNOSIS

The diagnostic approach of pyothorax is based on the clinical signs, thoracocentesis, the evaluation of the effusion and thoracic radiographs (MacPhail, 2007; Beatty and Barrs, 2010; Murphy and Papasouliotis, 2011a). Other medical imaging studies, such

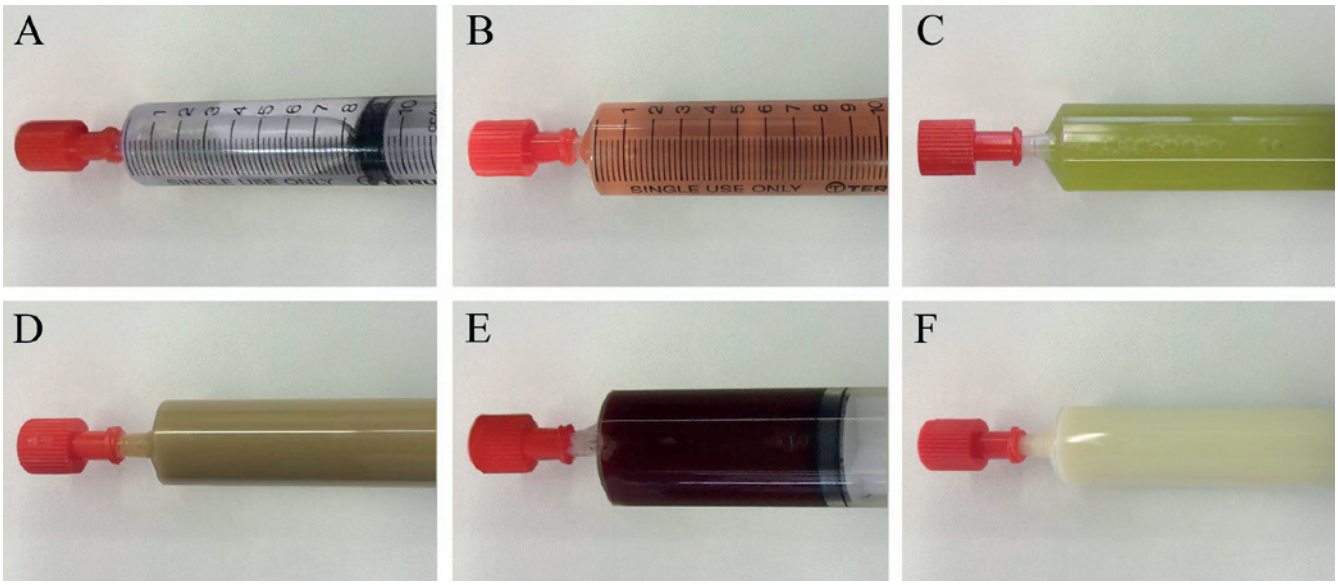

Figure 2. Macroscopic appearance of different types of effusion. A. Transudate secondary to protein losing enteropathy. B. Modified transudate caused by right-sided heart failure. C. Non-septic exudate caused by feline infectious peritonitis (FIP). D. Septic exudate from pyothorax. E. Hemothorax caused by mediastinal lymphoma. F. Idiopathic chylothorax.

as ultrasound (US) and computed tomography (CT), may be necessary to search for underlying causes. Complete blood count (CBC), serum biochemistry and urinalysis should form part of the minimum database to assess the general clinical condition of the patient and to guide the management. However, they are not crucial for the diagnosis itself (Beatty and Barrs, 2010). Cats should always be tested for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) (Waddell et al., 2002; Malik et al., 2006; Barrs and Beatty, 2009a).

Pleural fluid evaluation

The examination of pleural fluid is fundamental in the diagnostic work-up of animals with pleural space disease. Pleural effusion is classically divided in different categories based on protein content, total nucleated cell count (TNCC) and appearance on cytology (Ettinger and Feldman, 2010; Nelson and Couto, 2014) (Table 1). However, in one study, it has been suggested that Light's classification of pleural fluid in human medicine would be superior in cats as well. This classification is based on lactate dehydrogenase concentration in the pleural fluid, pleural fluid/serum lactate dehydrogenase ratio and pleural fluid/serum total protein ratio and is thought to classify transudate, modified transudate and exudate more accurately than the classical categorization, but further studies are needed (Zoia and Drigo, 2015). Aerobic and anaerobic culture should always be included (Love et al., 1982; Beatty and Barrs, 2010; Murphy en Papasouliotis, 2011a). The fluid should be collected in ethylene diamine tetra-acetic (EDTA) tubes for cell count and cytology, while a sterile container should be used for culture. For reliable anaerobic culture results, oxygen must be excluded from the transport specimen (Demetriou et al., 2002; Barrs and Beatty, 2009a).

In some cases, macroscopic evaluation may give a first indication towards the type of effusion (Figure 2). The purulent exudate in pyothorax is associated with a malodorous smell in up to 80% of cases (Piek and Robben, 2000; Waddel et al., 2002; Barrs et al., 2005; Barrs and Beatty, 2009a). The fluid is usually opaque and creamy, but it can also be pink, green tinged or serohemorrhagic. Flocculent particles are often present (MacPhail, 2007; Beatty and Barrs, 2010; Murphy and Papasouliotis, 2011a).

Cytological examination generally shows a large population of predominantly degenerate neutrophils, polymorphic inflammatory cells, a small proportion of mononuclear cells and large numbers of pleiomorphic, intracellular and/or extracellular bacteria (Demetriou et al., 2002; Barrs et al., 2005; MacPhail, 2007; Ettinger and Feldman, 2010). The macrophages demonstrate phagocytosis of debris and bacteria. In general, most cases of pyothorax are characterized by a polymicrobial infection on cytology (Waddell et al., 2000; Demetriou et al., 2002; Rooney and Monnet, 2002; Barrs et al., 2005; Klainbart et al., 2007). In one case series in 27 cats, 7% showed no bacteria on cytology, while 78% were presented with a polymicrobial infection and 15% showed a single type of bacterium (Barrs et al., 2005). In another case series in 14 cats and 36 dogs, no bacteria were seen in 20% of cases, 37.5% showed a polymicrobial infection and 42.5% was characterized by a single type of bacterium (Demetriou et al., 2002). Some bacteria, such as *Nocardia* spp. and *Actinomyces* spp., have a filamentous shape and acid-fast stains may aid in their differentiation (Demetriou et al., 2002; Malik et al., 2006; Doyle et al., 2009).

Infectious agents may not always be present cytologically due to prior antimicrobial therapy. The cytological results should therefore always be compared to the culture results (Barrs and Beatty, 2009a). Unfortunately, culture of pleural fluid may be false negative due to prior antibiotic therapy or insufficient growth of certain isolates in vitro. Positive bacterial cultures of pleural fluid have been reported in 68.7% of canine and feline cases with pyothorax (Demetriou et al., 2002). However, in other studies, a positive culture in less than half of the examined dogs has been reported (Johnson and Martin, 2007), whereas 78% of cats have a positive culture (Barrs et al., 2005). Low canine positive culture results may be explained by prior antibiotic therapy or by losing strictly anaerobic bacteria prior to culture, as a result of air contamination during sample collection and transport (Love et al., 1982; Piek and Robben, 2000; Johnson and Martin, 2007).

Complete blood count (CBC) and serum biochemistry

Complete blood count (CBC) generally shows a neutrophilic leukocytosis with a left shift, i.e. an increased concentration of nonsegmented or band neutrophils, as expected for pyogenic infections, but a degenerative left shift or leukopenia may be indicative for sepsis (Brady et al., 2000; Demetriou et al., 2002; Waddel et al., 2002; Barrs et al., 2005; Klainbart et al., 2007). A mild to moderate anemia is seen in up to 20% of feline and canine cases (Brady et al., 2000; Demetriou et al., 2002; Barrs and Beatty, 2009a). In 86% of feline cases with anemia, the anemia is nonregenerative and mostly normocytic and normochromic (Ottenjann et al., 2006).

The most common abnormalities on serum biochemistry are hypoalbuminemia, hyperbilirubinemia, hyponatremia, hypochloremia and mild elevations of aspartate aminotransferase (AST) (Barrs et al., 2005; Klainbart et al., 2007; MacPhail, 2007; Barrs and Beatty, 2009a). Hypoalbuminemia and hyperbilirubinemia are both common findings in sepsis (Brady et al., 2000). The decrease in albumin may be caused by increased vascular permeability, decreased hepatic synthesis and loss of protein in the pleural fluid in severe acute infections (Waddel et al., 2002; Barrs et al., 2005; Barrs and Beatty, 2009a). Hyponatremia and hypochloremia may be explained by decreased intake due to anorexia or may be attributed to the loss of fluid into the thoracic cavity. AST is commonly increased due to hepatocellular or myocyte damage. Possible mechanisms include hypoxia-induced damage secondary to poor perfusion due to hypovolemia or sepsis, inflammation from concurrent processes, e.g. pancreatitis, and infection within the liver caused by hepatic abscesses (Waddel et al., 2002).

Medical imaging

The importance of gentle handling of animals in respiratory distress cannot be overemphasized. Some procedures, such as medical imaging, may need to wait until the patient is stable enough, e.g. after thoracocentesis. Severe hypoxemia may occur if the animal is placed in lateral or dorsal recumbency. Reducing oxygen requirements and stress through minimal handling or fixation, combined with anxiolytic drugs and/or sedation and supplementation of oxygen are the first steps in stabilization to obtain better respiratory comfort (Beatty and Barrs, 2010).

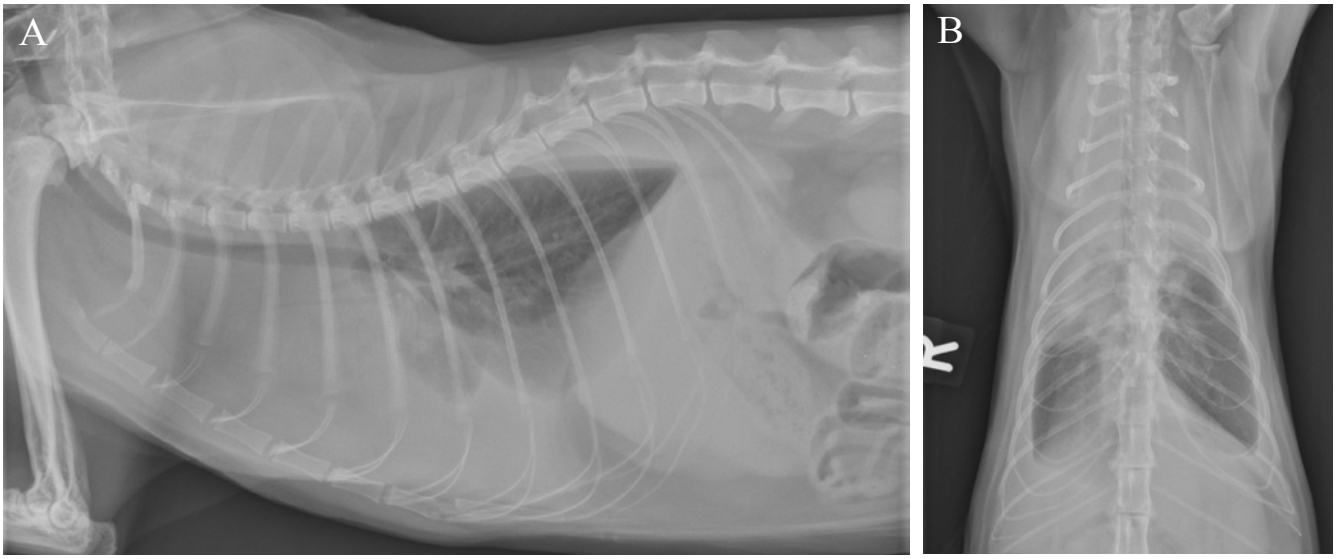

Figure 3A. Right lateral and dorsoventral thoracic radiographs of a cat with severe pyothorax. B. The radiographs were taken after left-sided thoracocentesis, through which 180 mL of yellow, opaque fluid had already been removed. The lung lobes are retracted from the thoracic wall by a soft tissue opacity in both left and right hemithorax. The effusion is asymmetrical, showing more severe effusion in the right hemithorax, which might be a consequence of previous left-sided thoracocentesis. There is scalloping of the ventral lung lobes.

Radiography (RX)

A single dorsoventral projection confirms the presence of pleural effusion (Beatty and Barrs, 2010), while a lateral projection helps to detect loculations (Christie, 2010). If the volume of effusion is small and more information is desired, other projections may be indicated. A ventrodorsal radiograph is more sensitive for the detection of small-volume effusions. However, there is a considerable risk of serious respiratory compromise if a patient has a moderate- to large-volume effusion. Therefore, this projection is not routinely advised (Murphy and Papasouliotis, 2011a). To assess if there is any underlying bronchopulmonary disease present, a complete set of thoracic radiographs should be obtained after pleural fluid removal, because they may have been effaced by fluid or obscured by atelectasis (Barrs and Beatty, 2009a; Beatty and Barrs, 2010; Epstein, 2014). It can take some time for the lungs to re-expand fully; hence, taking radiographs immediately after fluid removal may not be ideal (Murphy and Papasouliotis, 2011a).

Radiographically, a small volume of pleural effusion is characterized by the presence of interlobar fissure lines, though this may also be caused by pleural thickening (Murphy and Papasouliotis, 2011a). In cases with moderate to large amounts of free fluid, the retraction of the lobar borders from the thoracic wall, resulting in rounded lung borders, is particularly obvious in the caudodorsal areas of the lung. Other signs include lung collapse due to incomplete expansion, dorsal displacement of the trachea, widening of the mediastinum, obscuring of the cardiac silhouette and diaphragm, and scalloping of the lung margins at the sternal border (Barrs and Beatty, 2009a; Beatty and Barrs, 2010; Murphy and Papasouliotis, 2011a) (Figures 3A and B).

In dogs and cats, communication between the left and the right hemithorax may vary individually and can be influenced by concurring disease (Epstein, 2014). In a case study of 76 cats, a bilateral pleural effusion in 76%, a unilateral left-sided pleural effusion in 16% and a unilateral right-sided pleural effusion in 8% were reported (Barrs et al., 2005). The presence of unilateral effusion on radiographs should in any case raise the index of suspicion for pyothorax (or chylothorax) (Beatty and Barrs, 2010). Overall, cats with pyothorax have a higher frequency of unilateral effusion with up to 29% of cases, compared to 14% of cases in dogs with pyothorax (Demetriou et al., 2002; Barrs et al., 2005).

Ultrasonography (US)

Although thoracic radiography is more sensitive than ultrasonography in detecting small-volume pleural effusions, thoracic ultrasonography is a less invasive technique for the confirmation of a moderate to large volume of pleural effusion (Beatty and Barrs, 2010). Thoracic ultrasonography may also be indicated to identify consolidated lung masses, mediastinal masses and abscedated or neoplastic lung nodules. It can also be used for guided thoracocentesis when only a small amount of pleural fluid is present (MacPhail, 2007). The exudate in pyothorax is hypoechoic or complex echoic (Beatty and Barrs, 2010).

Computed tomography (CT)

With a computed tomography (CT) scan, the severity and the location of the pleural effusion can be determined and a detailed assessment of underlying parenchymal and pleural abnormalities can be provided (Swinbourne et al., 2011). In cases of migrating intrathoracic grass awns in dogs, CT has been reported to detect more sites of abnormalities and traces the foreign body pathway more accurately than radiographs (Swimbourne et al., 2011; Jiménez Peláez and Jolliffe, 2012; Vansteenkiste et al., 2014). Currently, CT is mostly used after patient stabilization to determine whether surgical intervention is indicated (MacPhail, 2007; Swimbourne et al., 2011). In contrast to what is mostly assumed, CT does not necessarily require general anesthesia and could therefore be used in more critical phases of diagnosis as well. While dogs require at least a deep sedation, cats tend to be fixated very well in a transparent container, e.g. VetMouseTrap, which allows quick and safe scanning without sedation (Oliveira et al., 2011; Schwarz and O'Brien, 2011).

TREATMENT

In some studies, death has been reported during clinical examination or shortly after (Mellanby et al., 2002; Barrs et al., 2005), highlighting the importance of minimal, careful handling and immediate supplementary oxygen (Barrs and Beatty, 2009b). The emergency patient should receive immediate intravenous fluid therapy if indicated. Afterwards, the level of pain should be assessed. Pleuritis and thoracic visceral pain are associated with a moderate to severe level of pain and a multimodal approach is advised (Lemke and Dawson, 2000; Mathews et al., 2014). In many cases, opioids are the initial drug of choice, e.g. buprenorphine; 0.01-0.02 mg/kg IV tid-qid. However, caution should be taken in patients with respiratory distress (Mathews et al., 2014). After stabilization, non-steroidal anti-inflammatory drugs, e.g. meloxicam; 0.1 mg/kg IV sid in dogs and 0.05 mg/kg SC in cats, can be added if there are no contraindications. Additionally, a CRI of ketamine, e.g. bolus of 0.5-1 mg/kg and CRI at 0.12-0.6 mg/kg/h in dogs; bolus of 0.5 mg/kg and CRI at 0.3-1.2 mg/kg/h in cats, may help in controlling severe pain (Mathews et al., 2014). If persisting thoracic visceral pain is suspected, the use of intrapleural blocks can be considered (Lemke and Dawson, 2000; Mathews et al., 2014). Treatment with systemic antibiotics alone usually does not over-

come the infection and removal of the exudate will be necessary (Piek and Robben, 2000). This drainage can take place through single or multiple thoracocenteses or though placement of thoracostomy tubes, with or without lavage of the pleural cavity (Piek and Robben, 2000; Demetriou et al., 2002; Rooney and Monnet, 2002; Boothe et al., 2010). In patients that are medically managed for two to three days without improvement, surgery should be considered (Monnet, 2009).

Antimicrobial therapy

Initial antimicrobial therapy is based on the cytologyof the pleural fluid. Single antimicrobial therapy in dogs has a 35%-risk of inefficacy. Therefore, a combined antimicrobial treatment seems prudent (Demetriou et al., 2002; Barrs and Beatty, 2009b; Boothe et al., 2010). A gram-stain of the fluid sample should be made and may help the clinician in choosing an appropriate antimicrobial agent for initial treatment (Love et al., 1982; Murphy and Papasouliotis, 2011b). Therapy should be altered afterwards, based on the results of culture and susceptibility testing (Walker et al., 2000; Klainbart et al., 2007; Barrs and Beatty, 2009b; Murphy and Papasouliotis, 2011b). Initial antibiotics should be administered parenterally, preferably intravenously. Once the patient is eating well, oral antibiotics may be substituted (Barrs and Beatty, 2009b).

Given that the majority of cases is characterized by synergistic polymicrobial infections caused by oropharyngeal flora, antibiotics should ideally be effective against anaerobes as well as gram-positive and gram-negative aerobes (Walker et al., 2000; Barrs and Beatty, 2009b). Penicillins and their derivates, e.g. amoxicillin-clavulanic acid, 10-40 mg/kg bid or tid, are reliably effective against obligate anaerobes, such as *Bacteroides* spp., and are especially a good treatment in cats, as enterobacteriaceae are not frequently isolated in the pleural fluid (Demetriou et al., 2002; Barrs et al., 2005; Barrs and Beatty, 2009b). Alternatively, fluoroquinolones, e.g. enrofloxacine, 5-7 mg/kg sid, or cephalosporins, e.g. cefazolin, 20-30 mg/kg tid, could be used as first-choice antibiotics (Demetriou et al., 2002; Greene, 2006; Barrs and Beatty, 2009b). Monotherapy with pradofloxacin has a high activity against isolates of anaerobic bacteria in dogs as well as in cats, but should be used with care because of the increasing resistance in treating human anaerobic infections (Stein and Goldstein, 2006). A combination of β-lactam antibiotics with fluoroquinolones for more than six weeks is advised to treat *Actinomyces*-infections (Sivacolundhu et al., 2001; Rooney and Monnet, 2002; MacPhail, 2007). Other antibiotics that can be used against *Actinomyces* spp. are clindamycin, chloramphenicol and gentamicin (Sivacolundhu et al., 2001; MacPhail, 2007; Barrs and Beatty, 2009b).

Metronidazole (15-50 mg/kg bid) can be used because of its lipophilic qualities. It is well distributed throughout the body and diffuses well into abscesses (Johnson and Martin, 2007). It is mostly used in combination with other antibiotics, because it is only effective against anaerobic bacteria (Piek and Robben, 2000; Murphy and Papasouliotis, 2011b). In high dosages or when administered for a long period of time, which is often necessary in the treatment of pyothorax, neurological side effects such as generalized muscle weakness can be seen (Piek and Robben, 2000).

Sulphonamides, e.g. trimethoprim-sulphamethoxazole (TMP-SDX), 5-10 mg/kg trimethoprim and 25-50 mg/kg sulfamethoxazole sid, are effective for high percentages of *Nocardia* isolates (Peabody and Seabury, 1960; Yildiz and Doganay, 2006; MacPhail, 2007; Malik et al., 2006; Sullivan and Chapman, 2010; Murphy and Papasouliotis, 2011b). This dosage of TMP-SDX is often effective, but not always well tolerated, resulting in excessive salivation due to the bitter taste, vomiting and partial to complete anorexia. In cats, high dosages may induce anemia and neutropenia due to bone marrow suppression (Malik et al., 2006). In dogs, severe neurological signs, such as generalized muscle weakness, may occur (Piek and Robben, 2000).

The appropriate duration of treatment in veterinary patients with pyothorax has not been well studied, but should be long-term, i.e. at least 4-6 weeks. In cases with isolation of filamentous organisms, treatment must be continued longer, since these infections are associated with devitalized tissue and tend to relapse if therapy is discontinued prematurely (Demetriou et al., 2002; Barrs et al., 2005; Malik et al., 2006; MacPhail, 2007). Treatment is necessary for a minimum of three months and can be prolonged for as long as one year in patients with disseminated disease (Sivacolundhu et al., 2001; Yildiz and Doganay, 2006).

Thoracic drainage

Needle thoracocentesis

Thoracocentesis can be diagnostic as well as therapeutic (MacPhail, 2007). Single or repeated needle thoracocentesis can be performed prior to tube thoracostomy. The removal of as much of the fluid as possible gives considerable relief (Barrs and Beatty, 2009b). Typically, a 20- or 22-gauge needle or butterfly catheter, connected to an extension tube with a three-way stop valve, is used. Using a sterile technique, the needle is advanced into the pleural cavity at the level of the ventral third of the thorax, mostly in the seventh or eighth intercostal space (ICS), cranial to the rib. Multiple thoracocenteses spread in time are generally not recommended and often ineffective (Barrs et al., 2005; MacPhail, 2007). However, one study performed by Johnson and Martin (2007) in

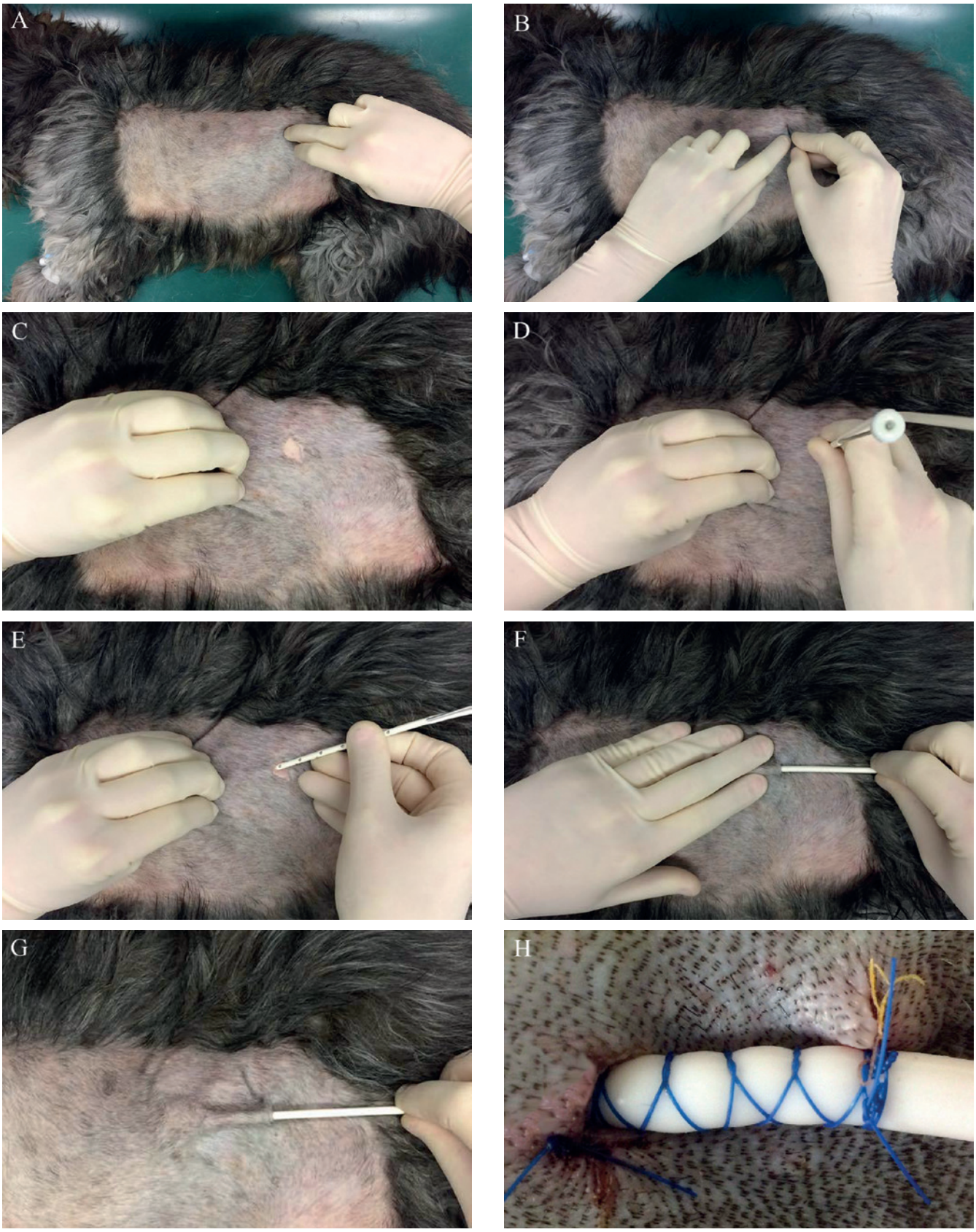

Figure 4. Placement and fixation of a thoracostomy tube in a dog. A. The eleventh intercostal space is localized. B. A skin incision is made in the dorsal third of the eleventh intercostal space. C. The skin is pulled cranially, bringing the skin incision at the level of the eighth intercostal space. D. The thoracic drain is inserted perpendicular to the thoracic wall. E. The thoracic drain is advanced in cranioventral direction. F. The skin is moved caudally. G. This results in subcutaneous tunnelling. H. The thoracic drain is attached to the skin using a purse-string suture and a Chinese finger trap.
Note: These pictures were taken on a euthanized dog. Sterile preparation and surgical draping are required to provide a sterile working field, but were disguarded in this case.

15 dogs without inhaled foreign body or pyogranulomatous effusion, showed a successful treatment of pyothorax in all 15 dogs through single unilateral thoracocentesis along with long-term antibiotic therapy.

Thoracostomy tubes and thoracic lavage

If needle thoracocentesis fails to stabilize or manage the clinical signs, chest drain placement is recommended (Valtolina and Adamantos, 2009). It can also be used in therapeutic procedures such as pleural lavage or in surgical cases following a thoracotomy (Murphy and Papasouliotis, 2011a). In complex cases with numerous pleural adhesions or in animals with a complete mediastinum, bilateral chest tubes are more likely to provide effective drainage than unilateral chest tubes (Barrs et al., 2005; Barrs and Beatty, 2009b).

The placement of thoracostomy tubes is simple and is generally well tolerated. Sedation or anesthesia may be necessary for uncooperative or stressed patients. The thoracostomy tube of the greatest diameter that can fit comfortably in the intercostal space (ICS) should be used, since wider bore tubes facilitate drainage of pus (Rahman and Gleeson, 2006; Barrs and Beatty, 2009b). The placement of chest tubes is preferably done with the animal standing or in sternal position (Frendin and Obel, 1997; MacPhail, 2007; Barrs and Beatty, 2009b; Murphy and Papasouliotis, 2011b). To minimize pneumothorax from leakage of air around the tube, subcutaneous tunneling of the drain should be achieved by entering the chest tube through the skin two or more ICS caudal to where the tube enters the thoracic cavity. After extensive clipping, aseptic preparation and local anesthesia, a small skin incision is made in the dorsal third of the tenth to twelfth ICS, lateral to the longissimus dorsi muscle. The skin is pulled cranially and the tube is inserted in the chest perpendicular to the thoracic wall around the eighth ICS. Afterwards, the drain is moved in craniocaudal direction. The tube needs to be clamped or a three-way valve has to be placed to prevent iatrogenic pneumothorax (Frendin and Obel, 1997; Barrs and Beatty, 2009b). Thoracostomy tubes without stylets can be placed, using large hemostats to perforate the intercostal muscles. After placement, tubes should be secured to the thoracic wall by a purse-string suture and a Chinese-finger trap to prevent the tube from slipping (Figures 4A-H). Finally, a bandage is needed to prevent the animal from manipulating the tube. The bandage should be changed at least once daily (Barrs and Beatty, 2009b; Murphy and Papasouliotis, 2011b). Control radiographs should be taken immediately after drain placement to assess its position. The tip of the drain should end in the ventral 2nd-3rd ICS to be positioned correctly (Barrs et al., 2005).

Chest tubes can be drained continuously or intermittently, e.g. every 4 hours. Continuous suction offers the advantage of maximal drainage, but gives more severe complications if the system detaches, which would remain unrecognized. Intermittent suction is easier, less expensive, requires less monitoring and is sufficient in most cases (Barrs and Beatty, 2009b; Murphy and Papasouliotis, 2011b). Hygienic procedures should be respected when draining the tube to prevent infection. Analgesia is necessary and usually includes systemic opioids. Interpleural use of bupivacaine is controversial. Some authors state that interpleural analgesia should be avoided in patients with poor respiratory reserves because of the potential diaphragmatic paralysis (Kowalski et al., 1992; Barrs and Beatty, 2009b). In other reports, it is described that, when using the correct dosages, e.g. 1.5 mg/kg, it can provide sufficient analgesia for up to eight hours with minimal risks of cardiovascular or pulmonary side effects (Lemke and Dawson, 2000; Glowaski, 2002).

Drainage should ideally be combined with intermittent thoracic lavage. This facilitates exudate drainage and prevents obstruction of the thoracostomy tubes by reducing pleural fluid viscosity. It also allows hydraulic debridement of the pleura, including breakdown of adhesions and dilution of bacteria and inflammatory mediators (Barrs and Beatty, 2009b; Boothe et al., 2010). Thoracic lavage must be carried out every four hours for the first two days. Afterwards, two to three times daily is usually adequate. As lavage solution, 0.9% sodium chloride or Hartmann's solution (to prevent hypokalemia), heated to body temperature, can be safely used in volumes of 10-25 mL/kg/lavage. Slow and hygienic infusion is necessary, combined with close monitoring of the patient. Recovery of 75% or more of the lavage solution after 30-60 minutes is expected, preferably after walking or moving the patient (Barrs and Beatty, 2009b; Boothe et al., 2010).

Complex loculated effusions or cases with advanced fibrinous or fibrous adhesions can be treated with subsequent administration of fibrinolytic agents through the tube (Rahman and Gleeson, 2006). Reported side effects include fever and bleeding. Fibrinolytic agents that can be used are heparin (10-15 IU/mL of lavage fluid), streptokinase, urokinase and tissue plasminogen activator (Demetriou et al., 2002; Boothe et al., 2010; Christie, 2010). Scientific evidence in veterinary medicine is still scarce, but in one study, improvement of both short- and long-term survival in dogs that were lavaged with a heparin-containing solution has been reported. No adverse effects were registered, but blood coagulation profiles were not monitored (Boothe et al., 2010).

In one study of 98 dogs, up to 22% of dogs developed some type of complication after the placement of thoracostomy tubes (Tattersall and Welsh, 2006). This is especially important in cases of pyothorax (and chylothorax), given that the tubes usually stay in place longer than in cases of other pleural space diseases. Therefore, complications occur more often (Tattersall and Welsh, 2006). Described complications include pneumothorax, serohemorrhagic dis-

charge from around the drain/skin interface, subcutaneous emphysema or edema, blockage of the drain with fibrin clots, infection of the thoracic wall with abscesses, lung tissue irritation or trauma, re-expansion pulmonary edema, arrythmias, phrenic nerve irritation and hemorrhage from laceration of intercostal vessels (Tattersall and Welsh, 2006; Barrs and Beatty, 2009b; Valtolina and Adamantos, 2009). In cats, the incidence of complications after thoracostomy tube placement is even higher, with a reported prevalence of 58% (Barrs et al., 2005).

Constant monitoring to observe changes in respiratory pattern and frequent clinical examination are advised. The volume of lavage solution administered and aspirated should be noted carefully and daily cytology of the fluid is recommended to assess therapeutic response. Regular monitoring with thoracic radiographs, preferably every two or three days, is necessary to detect failure of drainage due to incorrect tube placement, tube kinking or adhesions (Barrs and Beatty, 2009b; Murphy and Papasouliotis, 2011b). Thoracostomy tubes are generally removed after four to six days, but the ideal time of removal should be evaluated individually. Factors indicating possible removal are the reduction of the pleural effusion to 2-4 mL/kg/day, minimal amounts of remaining pleural effusion on thoracic radiographs and resolution of infection on cytology. Cytological examination of the pleural fluid should gradually contain less bacteria and less neutrophils with decreasing degenerative appearance (Demetriou et al., 2002; Klainbart et al., 2007; Barrs and Beatty, 2009b; Marques et al., 2009; Murphy and Papasouliotis, 2011b).

Surgical approach

The advantage of surgical treatment lies in a thorough exploration and removal of the primary cause with lavage and debridement of the pleural space. However, this must be weighed against the risks of general anesthesia in a compromised patient, the increased costs and prolonged hospital stay (Doyle et al., 2005; MacPhail, 2007). Common indications for early surgical intervention are the detection of an underlying lesion, e.g. abscess, foreign body, extensively loculated effusions or poor response to medical treatment after two to seven days (Rooney and Monnet, 2002; Barrs and Beatty, 2009b; Boothe et al., 2010). Surgery is also indicated when pneumothorax or drain obstruction caused by pleural adhesions, develops (Barrs and Beatty, 2009b). In dogs, a surgical approach is recommended if *Actinomyces* spp. is isolated, because of the poor outcome associated with medical therapy only, given the frequent association with migrating grass awns (Rooney and Monnet, 2002; Doyle et al., 2009). In cats however, the medical treatment of pyothorax caused by *Actinomyces* spp. (in combination with other oropharyngeal flora) is often sufficient, because it is less likely to be associated with grass awn foreign bodies (Barrs and Beatty, 2009b).

Intercostal thoracotomy or median sternotomy

Because surgical treatment of pyothorax usually requires exposure and exploration of both hemithoraces, median sternotomy is the most common surgical approach. It is used when preoperative workup reveals generalized disease or when no clear underlying cause can be found (Tattersall and Welsh, 2006; MacPhail, 2007; Boothe et al., 2010). Intercostal thoracotomy is not commonly used for exploratory purposes, but it may be a good approach when preoperative diagnostics reveal a focal lesion in a specific region (Tattersall and Welsh, 2006). It also enables more accurate positioning of thoracostomy tubes than median sternotomy (MacPhail, 2007; Barrs and Beatty, 2009b; Crawford et al., 2011). Affected lung tissue can be removed by pneumectomy. Acute loss of up to 50% of lung tissue is followed by compensatory changes in the contralateral lung. The removal of the entire right or left lung is usually well tolerated in cats. However, in dogs, right-sided pneumectomy is not well tolerated, because the right lung accounts for 58% of all lung tissue. Samples for bacteriology and, if indicated, histology must be taken during the surgical procedure. Postoperative oxygen supplementation, analgesia, careful monitoring and management of chest drainage are essential for successful recovery (Crawford et al., 2011).

Thoracoscopy

Video-assisted thoracoscopic surgery (VATS) is a recent diagnostic and therapeutic tool, which provides minimal invasive access to the thoracic cavity. It allows exploration of the entire pleural space, biopsies and culture samples, and debridement of the mediastinum and other tissues involved in the infectious process (Kovak et al., 2002; MacPhail, 2007; Monnet, 2009; Jiménez Peláez and Jolliffe, 2012). The disadvantages include the need for specific instrumentation and possible technical difficulties. Although there is little scientific evidence in veterinary medicine, thoracoscopy seems to be a safe and effective procedure in dogs and cats with rapid patient recovery, high success rates with shorter duration of chest tube drainage, less postoperative pain and shorter hospital stay than more invasive surgery (Christie, 2010; Jiménez Peláez and Jolliffe, 2012). If thoracoscopic exploration reveals multiple adhesions with severe involvement of lung lobes or pericardium, the conversion from thoracoscopy to sternotomy is advised (Monnet, 2009).

PROGNOSIS

The prognosis in cats and dogs with pyothorax is variable, ranging from excellent to extremely guarded, often resulting in death or euthanasia (Murphy and Papasouliotis, 2011a). The underlying cause, the extent of the disease and the rate of progression have an influence on both clinical signs and prognosis. Medi-

cal treatment fails in up to one third of all patients with pyothorax, but guidelines to when surgical intervention should be performed remain unclear and surgery is often disguarded due to financial concerns (Waddell et al., 2002; Rahman and Gleeson, 2006; MacPhail, 2007; Boothe et al., 2010).

One of the most common complications of pyothorax is recurrence (Demetriou et al., 2002; Waddell et al., 2002; Barrs et al., 2005). Recurrence rates are usually low, but vary greatly according to the type of treatment and the underlying cause (Demetriou et al., 2002; Rooney and Monnet, 2002; Waddell et al, 2002; Boothe et al., 2010). Cases involving *Nocardia* spp. or *Actinomyces* spp. tend to relapse most frequently, especially when treated without surgery, because they are often associated with complex pyogranulomatous disease (Peabody et al., 1960; Sivacolundhu et al., 2001; Malik et al., 2006; Doyle et al., 2009).

In cats, 50-100% of the non-survivors die or are euthanized within the first 48 hours after presentation. It is therefore considered that survival of the first 48 hours can serve as a good prognostic indicator (Demetriou et al., 2002; Waddell et al., 2002). Survival rates vary greatly according to the type of treatment. In one study of 80 cats, 66% of the cats survived, each of them receiving an appropriate type of treatment (Waddell et al., 2002). In 19 cats treated with intravenous fluids combined with antimicrobial therapy and drainage through thoracostomy tubes, a 95% success rate has been reported (Barrs et al., 2005). In contrast, mortality rates as high as 80% have been reported in cats when drainage was achieved through single or multiple thoracocenteses, without placement of chest tubes (Bauer, 1986). On average, cats that have undergone surgery, are generally hospitalized for six days prior to surgical intervention. They have higher survival rates than cats that have been treated more conservatively, probably due to more effective drainage postoperatively (Waddel et al., 2002). The available data in cats shows a recurrence rate between 5 and 8% in general, but it could increase up to 23% in cases of pyothorax caused by *Nocardia* infections (Demetriou et al., 2002; Waddell et al., 2002; Barrs et al., 2005; Malik et al., 2006).

In dogs, a good outcome is often seen when therapy includes intravenous antibiotic therapy and drainage of the pleural fluid, with or without the placement of thoracostomy tubes and lavage (Piek and Robben, 2000; Demetriou et al., 2002; Johnson and Martin, 2007). It should be noted that in these cases, foreign bodies or filamentous bacteria are usually not included (Piek and Robben, 2000; Demetriou et al., 2002; Johnson and Martin, 2007). Short-term survival rates of dogs undergoing surgical therapy are thought to be about five times higher than dogs treated conservatively (MacPhail, 2007; Boothe et al., 2010). Survival rates vary between 29 and 100% for medical treatment and up to 92% for surgical treatment (Mellanby et al., 2002; Rooney and Monnet, 2002; Boothe et al., 2010; Lee, 2014).

DISCUSSION

Pyothorax is an uncommon disease in dogs and cats, but can potentially be life-threatening. At the moment, there is no data available concerning the actual incidence of pyothorax in dogs and cats, but it appears to occur more frequently in cats (Demetriou et al., 2002; Rooney and Monnet, 2002; Barrs et al., 2005; MacPhail, 2007; Boothe et al., 2010). An immediate and appropriate diagnostic and therapeutic approach is essential to obtain a good outcome (Barrs and Beatty, 2009b; Firth and Boag, 2012). The time between the occurrence of the clinical signs and the start of therapy are of great importance. Treatment should minimally consist of careful handling, supportive care, antibiotic treatment and drainage of the pleural fluid (Rooney and Monnet, 2002; Barrs et al., 2005; Boothe et al., 2010). It is recommended to place bilateral thoracostomy tubes in all bilateral cases of pyothorax, although there is no clear concensus in the literature as to whether bilateral drainage is superior to unilateral drainage. In cases with multiple loculations of fluid, however, bilateral thoracostomy tubes seem to be necessary for adequate drainage of the pleural fluid (Demetriou et al., 2002; Barrs and Beatty, 2009b; Boothe et al., 2010; Christie 2010; Epstein, 2014; Lee, 2014).

Careful clinical and radiographic monitoring is important to assess therapeutic response. There is no clear data available as to whether or not surgery is indicated. However, it should be considered when there is poor reponse to medical therapy, in the presence of structural lesions or pneumothorax and in cases where involvement of filamentous organisms is suspected (Doyle et al., 2009; Murphy and Papasouliotis, 2011b). Thoracoscopy seems to be a very promising technique that can be used both diagnostically and therapeutically (Kovak et al., 2002; MacPhail, 2007). In some cases, with thoracoscopy, the cause of pyothorax can be resolved, but conversion to thoracotomy or sternotomy may still be necessary (Monnet, 2009).

A good first choice of antibiotic therapy consists of amoxicillin-clavulanic acid (10-40 mg/kg bid or tid) in cats and a combination of amoxicilline (20-40 mg/kg bid or tid) and enrofloxacine (5 mg/kg sid) should be considered in dogs, based on the susceptibility of the most commonly isolated organisms in both species (Greene, 2006). It should be emphasized that the appropriate antibiotic therapy should always be altered according to the results of cytology and gramstain, and if necessary, changed again according to the results of the bacteriological examination and susceptibility testing.

The optimal duration of antibiotic therapy in cases of pyothorax still needs to be elucidated. A sufficient duration of treatment may prevent recurrence of infection, which is a common and serious complication. Finally, veterinarians must take effort in preventing pyothorax by adequately treating bite wounds and local infections. Given that oropharyngeal bacteria are

the most common source of infection of the pleural space, it is recommended that cats undergoing dental surgery, cats suffering from upper respiratory tract infections and cats that have been involved in a catfight should be treated with antibiotics as a prophylactic measure (Barrs et al., 2005; Barrs and Beatty, 2009b). Whether this will actually reduce the incidence of pyothorax still needs to be investigated, and the potential benefits must be weighed against the risk of increasing antimicrobial resistance.

CONCLUSION

Pyothorax is thought to be an uncommon disease, but there is few data available regarding incidence, and most common underlying causes are yet to be further investigated. Treatment should always consist of supportive care, long-term antibiotics and drainage of the effusion, but it should be emphasized that there is no golden standard, and treatment approach should always be evaluated individually. Although the results of one canine study were promising, the addition of thoracic lavage is yet to be studied, as there is no clear scientific advice regarding the amount, the frequency and/or the type of lavage. In addition, further research regarding possible complications is indicated. Regarding the treatment of complex cases, video-assisted thoracoscopic surgery is a promising technique. However, further studies are needed to assess its advantages and disadvantages in comparison to more invasive thoracic surgery.

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GEBRUIK VAN YPOZANE ® BIJ DE REU

VRAAG

"Kan een reu melkklierontwikkeling krijgen door toediening van Ypozane®?"

ANTWOORD

Ypozane® (Virbac, België) bevat het hormoon osateronacetaat en wordt gebruikt als therapie voor benigne prostaathypertrofie (BPH) bij de reu (Virbac, 2007). Benigne prostaathypertrofie is een goedaardige vergroting van de prostaat waarbij zowel de grootte als het aantal epitheliale prostaatcellen toeneemt (Memon, 2007). Het ontstaat typisch bij oudere, intacte reuen door de inwerking van 5α-dihydrotestosteron, de metaboliet van testosteron. Onder invloed van 5α-dihydrotestosteron ontstaat zowel hypertrofie als hyperplasie van de epitheliale prostaatcellen met vergroting van de prostaat tot gevolg. Deze prostatomegalie leidt tot een verhoogde vascularisatie en een verhoogde druk, waardoor bloedingen en cysten ontstaan. Zolang de prostaat onder invloed blijft van androgenen blijft deze groei verdergaan (Moore et al., 1979).

Osateronacetaat vertoont een antiandrogene werking. Het verhindert enerzijds de opname van testosteron uit het bloed naar de prostaatcellen en gaat anderzijds een competitie aan met het 5α-dihydrotestosteron voor de androgeenreceptor in de prostaatcellen. Hierdoor wordt het prostaatvolume gereduceerd en verdwijnen de klinische symptomen geassocieerd met benigne prostaathypertrofie; namelijk bloed druppelen uit de penistop, incontinentie, hematurie, hemospermie, tenesmus, constipatie en diarree (Schotte et al., 2012).

Ypozane® is beschikbaar in tabletvorm voor honden in vier verschillende dosissen van 1,875 mg tot 15 mg. De medicatie wordt eenmaal per dag aan een dosis van 0,25 – 0,5 mg osateronacetaat per kg lichaamsgewicht toegediend gedurende zeven dagen. Een verbetering van de klinische symptomen vindt meestal plaats binnen de twee weken en het effect houdt aan gedurende tenminste vijf maanden (EMA, 2007). Na deze periode is het aangewezen om de hond door de dierenarts opnieuw te laten onderzoeken. Indien er eerder klinische symptomen ontstaan, wordt een vervroegd bezoek aan de dierenarts geadviseerd (Tabel 1).

Verschillende bijwerkingen worden gerelateerd aan het gebruik van Ypozane®. Zo komt in maximum 10% van de gevallen een tijdelijke verandering van de eetlust of hyperactief en erg aanhankelijk gedrag voor. Het optreden van braken, diarree, polyurie, polydipsie en lusteloosheid is zeer zeldzaam (Virbac, 2007).

Het gebruik van Ypozane® wordt tevens geassocieerd met melkklierontwikkeling. Deze bijwerking komt echter slechts bij 0,1 tot 1 % van de reuen voor. In zeer zeldzame gevallen (minder dan 1 op 10.000 dieren) gaat dit ook gepaard met lactatie (Virbac, 2007). Wanneer reuen plotseling aantrekkelijk worden voor andere reuen of wanneer de borstklieren in omvang toenemen, wordt dit een feminisatiesyndroom genoemd (Rijsselaere, 2011).

Al deze neveneffecten verdwijnen na enige tijd zonder specifieke behandeling, mits de toediening van Ypozane® wordt stopgezet (Albouy et al., 2008; Anonymous, 2010; Renggli et al., 2010).

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Tabel 1. Dosering Ypozane® (uit Virbac, 2007).

Gewicht van de hond	Hoeveelheid Ypozanetabletten	Aantal tabletten per dag	Behandelingsduur
3 tot 7,5 kg	1,875 mg Tablet	1 Tablet	7 Dagen
7,5 tot 15 kg	3,75 mg Tablet		
15 tot 30 kg	7,5 mg Tablet		
30 tot 60 kg	15 mg Tablet		

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ANTICONCEPTIE VOOR DUIVEN

VRAAG

"Blijkbaar is er nu medicatie om de voortplanting bij duiven te beperken. Kunt u enige info verstrekken? Bestaat het? Werkt het? Zijn er nevenwerkingen voor de duiven? Hoe toe te dienen? "

ANTWOORD

De aanwezigheid van grote aantallen stadsduiven vormt nog steeds een aanzienlijk probleem omdat hun uitwerpselen schade aan gebouwen veroorzaken, aanleiding geven tot onhygiënische situaties en gezondheidsrisico's voor de mens en andere wildlevende dieren met zich meebrengen (Johnston, 1992). Verschillende methoden werden reeds toegepast om aan de uitbreiding van de populatie van stadsduiven een halt toe te roepen, zoals het vangen en vernietigen van duiven, het vernietigen van duiveneieren of het vervangen van eieren door kunsteieren, sterilisatie van stadsduiven en het gebruik van afschrikmiddelen (MacDonald et al., 2013). Geen enkele van deze methoden heeft echter geleid tot een significante beperking van de duivenpopulatie tot een aanvaardbaar niveau (Albonetti et al., 2015).

Nicarbazine wordt sinds 1950 gebruikt als coccidiostaticum bij kippen (Jones et al.,1990). De bijwerking van deze stof bij vogels is dat de ontwikkeling van eieren verstoord wordt, afhankelijk van de toegediende dosis (Sherwood et al.,1956; Yoder et al., 2006). Aanvankelijk werd van deze eigenschap gebruik gemaakt om vrijlevende, residentiële populaties canadaganzen (*Branta canadensis*) in te tomen (Avery et al., 2008; MacDonald en Wolf, 2013). Nicarbazine werd reeds geregistreerd en gecommercialiseerd als contraceptief middel ter beperking van in het wild levende populaties duiven die voor overlast zorgen in de Verenigde Staten (OvoControl® P, Innolytics, San Clemente, VSA) en enkele Europese landen (Ovistop®, Acme srl., Cavriago, Reggio Emilia, Italië). Nicarbazine is nu eveneens geregistreerd in België onder de naam R-12® (Acme srl., Cavriago, Reggio Emilia, Italië) (FAGG, 2016). Het product R-12® is geformuleerd als een voedermengsel bestaande uit met nicarbazine geïmpregneeerde maïskorrels, is enkel op voorschrift verkrijgbaar en mag alleen toegediend worden door een dierenarts. Het toepassen van de behandeling dient onder andere afgestemd te worden op de dynamiek van de te bestrijden duivenpopulaties en daarom worden er door de houder van de registratie als geneesmiddel opleidingen voorzien (ACME, 2016).

Alhoewel er eensgezindheid lijkt te bestaan omtrent de anticonceptieve werking van nicarbazine bij vogels, valt er een grote variatie op wat betreft de posologie van Ovistop® enerzijds en die van OvoControl® P anderzijds. De aangewezen individuele dosis actief bestanddeel voor Ovistop® is namelijk opvallend hoger dan voor OvoControl® P. Niettemin zou een reductie op populatieniveau van 40 % en 53 % respectievelijk na vier (Albonetti et al., 2015; Giunchi et al., 2007) en één jaar (MacDonald en Wolf, 2009) moeten opgemerkt worden. In verschillende steden in Spanje en Italië werd R-12® reeds ingezet en werd na één jaar reeds een vermindering van 30 tot 70 % vastgesteld bij de behandelde duivenpopulaties.

Aangezien het anticonceptieve effect van nicarbazine bij vogels reversibel is (Yoder et al., 2005), vraagt een langdurige inperking van een populatieniveau eventueel herhaaldelijke toediening. Tot nu toe werden er geen nevenwerkingen gerapporteerd bij behandelde duiven. Er dient uiteraard wel rekening gehouden te worden met de beperkte mogelijkheid tot langdurige opvolging van de behandelde vogels (Giunchi et al., 2007). Daarom wordt er aangedrongen op directe rapportering van bijwerkingen aan de producent of het FAGG. De gevolgen van het gebruik van nicarbazine op de omgeving en andere wilde dieren is eveneens nog niet onderzocht.

Ondanks het ontbreken van betrouwbare gegevens omtrent toxiciteit lijkt er door het gebruik van nicarbazine een hogere efficiëntie bereikt te worden wat betreft het inperken van vrijlevende vogelpopulaties dan met andere methoden (Giunchi et al., 2007).

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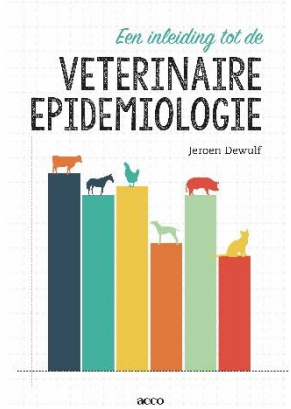

Inleiding in de veterinaire epidemiologie

Auteur: Jeroen Dewulf
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Dit boek geeft een praktisch overzicht van de voornaamste epidemiologische concepten in de diergeneeskunde. Het gaat over het voorkomen en de verspreiding van dierziekten, via test, evaluatie en studieopzet tot en met risicoanalyse en ‘evidence based veterinary medicine’. Praktische en reële voorbeelden tonen aan hoe epidemiologische concepten toegepast worden in de diergeneeskundige praktijk. Een inleiding tot de veterinaire epidemiologie is van onschatbare waarde voor iedere diergezondheidswerker die met diergeneeskundige vraagstukken wordt geconfronteerd en deze op een gefundeerde en onderbouwde manier wil aanpakken.

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