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UNIVERSITEIT GENT

- Stress bij slangen
- *Trypanozoma equiperdum* bij het paard
- Artroscopische bevindingen bij caniene ellebogen
- Chemotherapie bij een valkparkiet
- Bioveiligheid in de rundveeproductie
- IOHC bij de hond
- Romeinse hondengrafvondsten in Tongeren

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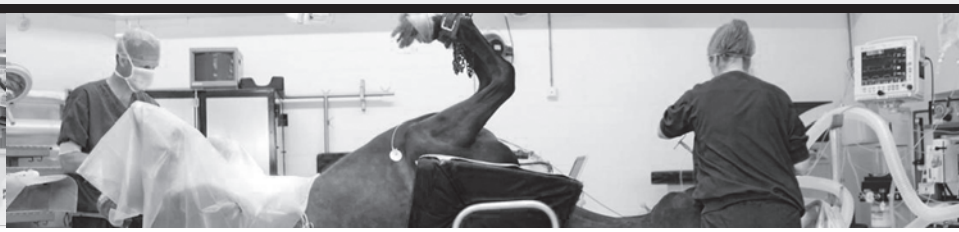

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Voor aanvullende informatie over deze vacature kun je telefonisch of per mail contact opnemen met mej. J. Benschop.

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VLAAMS DIERGENEESKUNDIG TIJDSCHRIFT

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INHOUD

Coverfoto: Gunther Antonissen

De **valkparkiet (*Nymphicus hollandicus*)** is een endemische vogelsoort uit Australië. Hij is een populaire kooi- en volièrevogel. De wetenschappelijke naam van de valkparkiet werd in 1792 gepubliceerd door Robert Kerr als *Psittacus hollandicus*. In 1832 werd de soort door Johann Georg Wagler in het geslacht *Nymphicus* geplaatst. *Nymphicus* betekent gelijkend op een nimf en *hollandicus* verwijst naar Nieuw-Holland, een oude benaming voor een deel van Australië. Onderzoek naar mitochondriaal DNA heeft aangetoond dat de valkparkiet geen echte parkiet (of papegaai) is, maar een kleine kaketoe. Goedaardige en kwaadaardige tumoren komen zeer frequent voor bij deze gezelschapsvogels. De laatste decennia werd het gebruik van chemotherapie bij de vogels beschreven met variërend succes. Deze variërende resultaten zijn voornamelijk te wijten aan het empirische gebruik van de chemotherapeutica en het feit dat de behandelingsmethoden geëxtrapoleerd worden van deze gebruikt bij de hond en de kat.

Tekst: Gunther Antonissen

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Stress in wild and captive snakes: quantification, effects and the importance of management

Stress bij in het wild en in gevangenschap levende slangen: kwantificatie, gevolgen en het belang van management

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ABSTRACT

As in other animals, distress and impaired welfare have a deleterious effect on the mental, physical and behavioral health of snakes in the wild and in captivity. Besides anthropogenic disturbance, the availability of food and shelter, the presence of predators, and environmental factors, such as seasonality and climatological changes, are important factors that affect the stress level and subsequent welfare in wild snake populations. In captive snakes, inappropriate management is the most prominent cause of chronic stress and impaired welfare. Chronic stress can be assumed by looking at the snake's behavior, but there is need for a standardized quantification method to pin-point more accurately (chronic) stress levels. The biomarker suitable in this framework is the level of corticosterone in plasma, feces and shed skin.

SAMENVATTING

Net zoals bij andere diersoorten hebben stress en verminderd welzijn een negatief effect op de mentale status, gezondheid en op het gedrag van zowel in het wild als in gevangenschap levende slangen. Naast antropogene verstoring zijn de beschikbaarheid van voedsel en schuilplaatsen, de aanwezigheid van predatoren en omgevingscondities, zoals seizoenale en klimatologische veranderingen, de belangrijkste factoren die stress en welzijn van wilde slangenpopulaties beïnvloeden. Bij in gevangenschap levende slangen is een ongeschikt management de meest prominente reden van chronische stress en verminderd welzijn. Chronische stress kan bepaald worden door gedragswijzigingen van de slang, maar een gestandaardiseerde kwantificatiemethode om (chronische) stresslevels accurater te bepalen, ontbreekt. Een biomerker die gebruikt kan worden, is het gehalte aan corticosterone in bloedplasma, feces en vervellingshuid.

INTRODUCTION

All vertebrates respond to unpredictable and stressful conditions with the so-called 'stress response' (Wingfield, 2005). Stressors can be defined as stimuli that put the individual in a state of uncertainty, lack of information and/or lack of control (Brown et al., 1991). Snakes faced with such stressful stimuli launch a stress response, not only through secretion of catecholamines, such as adrenaline and noradrenaline into the blood, but also through an endocrine stress response with activation of the hypothalamic-pituitary-adrenal (HPA-) axis, to release glucocorticoids, in particular corticosterone into the blood (Sapolsky et al., 2000) (Figure 1). Corticosterone is analogous to cortisol, a glucocorticoid present in mammals, and can be seen as an adaptive hormone as it facilitates energy availability to body systems through gluconeogenesis in order to face the stressor(s) (Palacios et al., 2012; Silvestre, 2014; Tarlow and Blumstein, 2007). A stressor may be acute, eliciting an acute short-termed response causing physiological and behavioral changes that counteract the stressor. This is only temporarily and allows the individual to quickly return

to its normal activities (Dantzer et al., 2014; Dickens and Romero, 2013). In contrast, a chronic, sustained response with non-adaptive levels of glucocorticoid secretion, will disturb chronically normal activities, leading to an impaired welfare (Claunch et al., 2017; Dantzer et al., 2014; Davis et al., 2008; Dickens and Romero, 2013; Tarlow and Blumstein, 2007).

Determination and quantification of stress are pivotal because of its relation to the general performance and health of the individual. Behavioral changes indicate disturbance, injury or disease, but may also lead to physical lesions and infections if prolonged. Higher corticosterone levels caused by chronic stress lead to immune suppression, making the individual more susceptible to viral, bacterial and parasitic infections (Dantzer et al., 2014; Neuman-Lee et al., 2015; Silvestre, 2014). However, the perception of stressors is highly individual; whereas one individual does not experience stress from a specific stimulus, the welfare of another individual could be strongly impaired. This individual perception is caused by intrinsic factors, such as sex, age, reproductive condition and former experiences (Dantzer et al., 2014) (Figure 2).

A plethora of stimuli may influence the welfare of snakes. The most common stressors for snakes in the wild are food deprivation, habitat loss due to anthropogenic activity and changes in climate (Ajtić et al., 2013; Gregory, 2016; Robert and Bronikowski, 2009). Other potential stressors are the presence of predators and non-indigenous species in the same habitat (Burger, 1998; Gregory, 2016; Pasmans et al., 2017; Šukalo et al., 2014). Snakes in captivity are mainly stressed by management related aspects, such as temperature, humidity, enclosure size and crowding (Silvestre, 2014).

The quantification of chronic stress in a wild snake population is pivotal for their managment and habitat, while for snakes in captivity, it is important regarding welfare. However, this is challenging due to the individual nature of the chronic stress response, but also because of the lack of a uniformly used and standardized quantification method and matrix in the pertinent literature. In the literature, various matrices such as blood, feces and shed skin have been described to measure corticosterone as a biomarker for chronic stress, but comparing results of these studies using different methods and matrices is challenging (Dantzer et al., 2014; Sheriff et al., 2011). In the last section, the commonly used matrices to quantify corticosterone, i.e. blood, feces and shed skin are discussed.

STRESS IN WILD SNAKES

Over the last decade, the welfare of captive as well as wild animals has become increasingly important. In this framework, Bonnet et al. (2016) studied the rapid pace of urbanization and its negative impact on biodiversity, the functioning of ecosystems and the subsequent impairment of animal welfare in snakes. Assessment of stress in wild snake populations is challenging and has been underexamined. However, having a good understanding of stressful stimuli and their effects on welfare impairment is needed in managing snake populations.

The direct effect of food resources on the stress level has been little studied; however, it is known that they influence the size of a population. In a study by Sewell et al. (2015), the population size of grass snakes (*Natrix natrix*) positively correlated with common frog (*Rana temporaria*) spawn clumps and peak counts of pool frogs (*Pelophylax lessonae*) on a site in eastern England restored for the reintroduction of these amphibians. This was explained by the migration of snakes when food resources in this site increased. Management of forests and heathland which damages prey species populations, has been shown to have a negative impact on the preservation of wild snake populations (Reading and Jofré, 2013).

Secondly, loss of habitat caused by urbanization also affects wild snake populations. Dantzer et al. (2014) have shown that anthropogenic disturbances are frequently correlated with increased levels of plasma baseline glucocorticoids. Bonnet et al. (2016) has shown that shrub habitats, being important shelters for several snake species, have been dramatically altered for practical and esthetical reasons. This shift results in a decrease of sufficient habitat for snake species, which in turn has a negative effect on their population size and biodiversity. In addition, the presence of snakes sometimes triggers rather negative perceptions by the public, making local authorities remove snakes

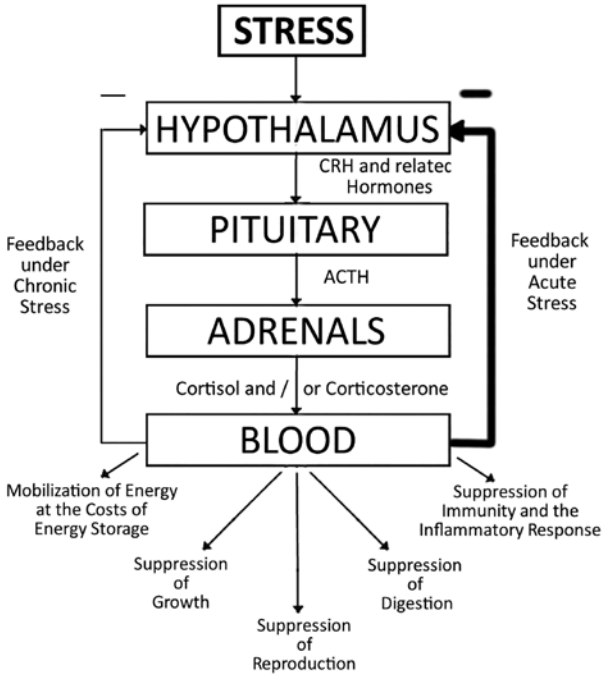

Figure 1. HPA-axis and its effect on systemic processes and the negative feedback response under acute stress compared with response under chronic stress (Sheriff et al., 2011).

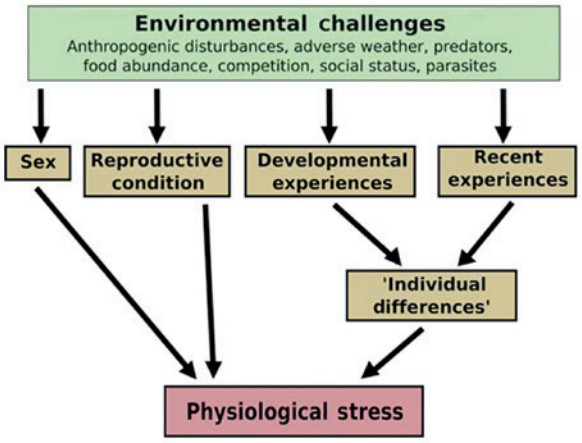

Figure 2. Influence of the intrinsic factors of the individual on its stress response (Dantzer et al., 2014).

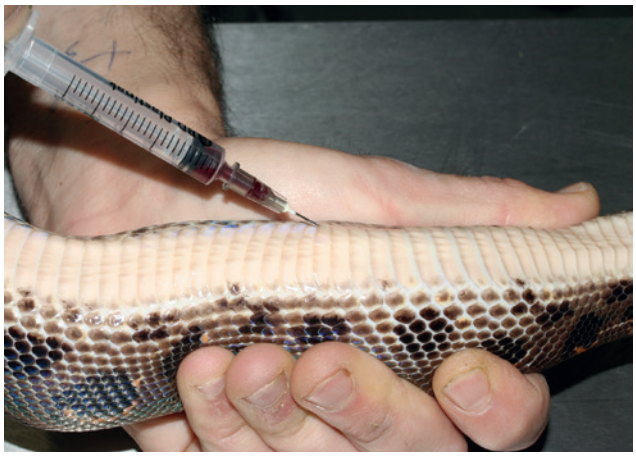

Figure 3. Blood collection from the heart of a snake.

from parks and green areas (Ballouard et al., 2013). For example, changes in French legislation in 2007 allowed the clearing of the aspic viper (*Vipera aspis*), a species previously marked as 'protected' (Bonnet et al., 2016).

Lastly, climatological changes are believed to have an impact on the overall performance of wild snake populations. Snakes, like all reptiles, are ectothermic, relying on the environment to maintain their body temperature. Snakes living in the temperate-zone are well-adapted to harsh wintering conditions as they hibernate. During this dormant period, their body temperature is generally lower than during active periods in contrast to plasma corticosterone, which is generally higher during hibernation (Dupoué et al., 2013; Nordberg and Cobb, 2016). Subsequently, it has been assumed that mild winter conditions trigger a state of chronic stress and a decrease of body condition when emerging. However, in a study by Brischoux et al. (2016), lower levels of plasma corticosterone were demonstrated when hibernation temperatures were higher, even though there was more body mass loss in snakes maintained in mild wintering conditions. Increased body mass loss may be caused by higher metabolism resulting from significant energy use during mild wintering conditions (Brischoux et al., 2016). Furthermore, it is possible that the experimental design of the study and the matrix, in which corticosterone was measured, affected the quantification of corticosterone. Therefore, further studies must be conducted to assess the effect of global warming on chronic stress levels.

STRESS IN CAPTIVE SNAKES

Inappropriate management conditions are the main reason of stress and impaired welfare in snakes in captivity (Silvestre, 2014). Factors like temperature, humidity, enclosure size and crowding all influence snake welfare, hereby restricting the physiologic needs of the animals. Persistent inadequate conditions lead to chronic stress and, with time, to dwindling (DeNardo, 2006). Raising awareness of the effect of bad management on mental, physical and behavioral health is the key to improve snake welfare in captivity (Silvestre, 2014). In some countries, guidelines for husbandry standards for reptiles are available providing a good framework of management factors for snakes kept in captivity (Table 1).

In a study by Sparkman et al. (2014) captivity of wild gravid female snakes (*Tamnophis* Elegans) resulted in increased plasma corticosterone levels as well as heterophile to lymphocyte (H:L) ratios over time. Maladaptation syndrome is a phenomenon occurring in reptiles that are acquired from the wild but do not adapt to captivity, subsequently leading to their death (DeNardo, 2006).

Assessment of chronic stress

In the pertinent literature, chronic stress is measured in various ways (Tables 2 and 3). In some studies, the snake's behavior is observed, especially when studying snakes in captivity (Silvestre, 2014). If the snake is unable to flee from its stressor, which is mostly the case in captivity, the animal will be unable to respond in an appropriate manner and will therefore express abnormal, non-functional behavior (DeNardo, 2006; Silvestre, 2014). Behavioral change in captive reptiles may indicate disturbance, injury or disease (Warwick et al., 2013). These changes can be used to evaluate their condition and welfare, but must be considered carefully in their context. A normally active reptile may become lethargic due to stress, whereas an individual with a calm nature may become very active. Also physical lesions and topical infections, e.g. rostral lesions incurred by rubbing their heads against transparent boundaries, are regarded as indicators for impaired welfare. However, in a study by Claunch et al. (2017), corticosterone administered to the Southern Pacific rattlesnake (*Crotalus helleri*) by

Table 1. Management factors for five popular snake species bred in captivity (From: Belgian law of animal welfare, 2004; guidelines from German federal ministry of food and agriculture, 1997; LICG, 2017).

	Management of popular snake species		
	Boa constrictor (*Boa constrictor*) ([1]<4 m)	**Reticulated python (*P. reticulatus*) ([1]<10 m), Tigerpython (*P. molurus*) and African rock python (*P. sebae*) ([1]<7 -8 m)**	**Corn snake *(Elaphe guttata*)**
Temperature	Gradient; 20 – 35 °C, at night 20 – 22 °C	Gradient; 26 °C – 38 °C, at night 5 – 10 °C	Gradient; 22 – 32 °C, at night 18 – 20 ° C
Humidity	Moderate; 60-85%	High; 70 – 90%	Low; 40 – 60%
Light	12 hours/day	12 hours/day	12 hours/day
Stocking density	1 - [2]2 snakes per tank	1 - [2]2 snakes per tank	1 – 2 snakes per tank
Terrarium lay-out	Heating place, hiding places, swimming pool and climbing areas	Heating place, hiding places, swimming pool and climbing areas	Heating place, hiding places, small swimming pool and climbing areas. Hibernation recommended
Terrarium size per snake (length x width x height)	[1]Under 1,5m: 0.75 m x 0.5 m x 0.75 m [1]Above 1.5m: 1.0 m x 0.5 m x 0.75 m [2]2 m² surface with 1.5 m height	[1]Under 2.5 m: 0.75 m x 0.5 m x 0.5 m [1]Above 2.5m: 1.0 m x 0.5 m x 0.75 m [2]8 m² surface with 1.5 m height	[1]1.0 m x 0.5 m x 1.0 m [2]0.5 m² surface with 0.60 m height
Substrate	Paper, pebbles or woodchips	Paper, pebbles or woodchips	Paper, pebbles or woodchips

[1] Minimum standards according to German federal ministry of food and agriculture
[2] Standards for reptiles kept in zoos, according to the Belgian law of animal welfare, June 23, 2004.

an implant with 3.6 mg (snakes less than 800 g) or 6.1 mg (snakes of more than 800 g) did not mediate their behavioral trait expression. Therefore, observing snake's behavior might not be the best way to ascertain chronic stress.

Levels of circulating cortisol and corticosterone are widely accepted as biomarkers for stress in vertebrates. Both are pleiotropic having besides their role as a stress hormone, various functions in non-stress related physiological processes (Dantzer et al., 2014; Silvestre, 2014; Sparkman et al., 2014). In addition, individual differences in HPA-axis reactivity related to intrinsic factors, such as sex and age, make the interpretation of corticosterone levels challenging (Sparkman et al., 2014). Corticosterone can be determined by means of radioimmunoassay (Palacios et al., 2012; Sparkman et al., 2014), enzyme immunoassay in blood or skin (Berkvens et al. 2013), or by measuring fecal glucocorticoid metabolite (FGM) using the referred methods (Berkvens et al., 2013).

Plasma corticosterone is used to quantify acute stress in snakes. The samples can be obtained quickly, and only a small volume (ca. 25–50 µL) is required to quantify glucocorticoids (Tarlow and Blumstein, 2007) (Figure 3). The response time of plasma corticosterone to acute stress caused by capture and handling is fast; hence, biasing may occur when samples are not taken in time (Sparkman et al., 2014). Palacios et al. (2012) showed that a difference of approximately 300 ng/mL corticosterone can occur between blood sampling of garter snakes (*Thamnophis elegans*) at capture time and 50 minutes later. Nonetheless, an elevation in baseline blood corticosterone can be used to determine chronic stress (Dantzer et al., 2014; Palacios et al., 2012). An elevated baseline of blood corticosterone may be caused by acute stressful stimuli and/or the reduced ability to terminate the stress response. This principle is based on the assumption that chronically stressed individuals will initially respond greater to acute stimuli such as the blood sampling itself (Dantzer et al., 2014), which is an invasive method (Tarlow and Blumstein, 2007). In addition, plasma levels of corticosterone are influenced by circadian as well as other rhythmicities making plasma unsuitable for chronic stress quantification (Silvestre, 2014).

Measuring FGM levels is a way to assess HPA-axis activity over a specific time lapse, and has gained interest for the quantification of stress in multiple species. In snakes, specifically, fecal corticosterone metabolite (FCM) is measured (Berkvens et al., 2013). It is assumed to be a cumulative exposure of corticosterone reflecting the average of the blood corticosterone over a specific duration (Dantzer et al., 2014; Sheriff et al., 2011). Measuring FGM levels is non-invasive, and therefore not altering results due to sampling (Dantzer et al., 2014; Palme et al., 2005). However, this matrix has its limitations. In mammals and birds,

Table 2. Comparison of different criteria to score stress in reptiles (part 1). N/A = not assessed (Adapted from: Brischoux et al., 2016; DeNardo, 2006; Dickens and Romero, 2013; Fitze et al., 2009; Silvestre, 2014; Warwick et al., 2013).

Scoring stress in reptiles (in theory): part 1				
Criterium	**Behavioral change**	**Higher occurrence of lesions and infections**	**Change in color**	**Weight loss**
Specificity	Low	Low	Low	Low
Sensitivity	Low	High	Low	High
Quantification	Not possible	Not possible	Not possible	Not possible
Invasiveness	Non-invasive	Non-invasive	Non-invasive	Non-invasive
Applicability	Non-species specific	Non-species specific	Species specific	Non-species specific
Expertise needed	High	Moderate	High	Low
Validation	N/A	N/A	N/A	N/A
Biasing factors	Observer, intrinsic factors	Observer, other agents causing lesions and infections	Observer, dietary differences	Infections, dietary differences

dietary differences and metabolic rate have been shown to have an influence on FGM levels (Dantzer et al., 2011; Goymann et al., 2006). In a study of Dantzer et al. (2011), higher dietary fiber consumption seemed to increase fecal cortisol metabolite (FCM) concentration in North American red squirrels (*Tamiasciurus hudsonicus*). Moreover, microbial interconversion, degradation after defecation or wash-out by rainfall may biase results (Tarlow and Blumstein, 2007; Washburn and Millspaugh, 2002).

Although little studied, corticosterone in shed skin originated by ecdysis could be the future matrix for chronic stress quantification. Ecdysis is the process by which an old outer keratinized epithelial layer is shed, revealing new keratinized epidermal layers underneath (Maderson, 1965). Blood corticosterone is presumed to incorporate in the skin, but this level is possibly biased by extra-adrenal production as is seen in human skin (Taves et al., 2011). Berkvens et al. (2013) showed a positive correlation between fecal and shed skin corticosterone in African house snakes (*Lamprophis fuliginosus*) and Eastern Massasauga rattlesnakes (*Sistrurus catenatus catenatus*), indicating that the status of the HPA-axis over a specific time frame influences fecal and skin corticosterone in a similar manner. Just as in feces, chronic stress results in a higher cumulative exposure of corticosterone in skin; and as corticosterone in shed skin represents the status of the HPA-axis over a time lapse, it could be used to determine chronic stress (Dantzer et al., 2014; Sheriff et al., 2011). However, up till now, positively correlated levels of corticosterone in shed skin with

Table 3. Comparison of different criteria to score stress in reptiles (part 2). N/A = not assessed, ? = unknown. (Adapted from : Berkvens et al., 2013; Brischoux et al., 2016; Dantzer et al., 2014; Davis et al., 2008 ; Dickens and Romero, 2013; Kalliokoski et al., 2012; Silvestre, 2014).

Scoring stress in reptiles (in theory): part 2				
Criteria	**Heterophile/ Lymfocyte ratio (H:L ratio)**	**Corticosterone in plasma**	**Fecal glucocorticoid metabolite (FGM)**	**Corticosterone in shed skin**
Specificity	Low to ?	?	?	?
Sensitivity	? to high	?	?	?
Quantification	Possible	Possible	Possible	Possible
Invasiveness	Invasive	Invasive	Non-invasive	Non-invasive
Applicability	Species specific	Species specific	Possibly species specific	Species specific
Expertise needed	High	High	High	High
Validation	N/A	N/A	In iguana's	N/A
Biasing factors	Infections and inflammations	Intrinsic factors, acute stress, daily and seasonal cycles	Intrinsic factors, dietary differences, metabolic rate and degradation after defecation	Intrinsic factors, possibly extra-adrenal pathways

stressful environmental stimuli have not been reported yet (Berkvens et al., 2013; Sheriff et al., 2011).

CONCLUSION

Frequently, wild as well as captive snakes are confronted with chronic stress, affecting their overall performance and health. The quantification of stress in snakes, particularly of chronic stress, is challenging and quantification of the stress hormone corticosterone in different matrices, such as plasma, feces and skin, has its advantages and disadvantages. Regardless of the matrix used, the interpretation of results is difficult as the perception of stressors differs between individuals and is influenced by intrinsic factors, such as age and sex. Therefore, further research is needed to provide a scientifically validated quantification method for chronic stress in snakes.

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Uit het verleden

DE 'WORM' ALS ZIEKTEOORZAAK

Een eeuwenoud geloof schrijft allerhande ziekten toe aan geheimzinnig woekerende 'wormen' van demonische krachten voorzien. Hierbij moet men niet denken aan de ons bekende plat- en rondwormen, maar aan meestal onzichtbare ziekteverwekkers. Denk ook aan het Franse vermine (klein ongedierte, zoals bladluizen en rupsen), afgeleid van het Latijnse vermis, in feite hetzelfde woord als worm.

Vermoedelijk is dit geloof ontstaan vanuit de observatie van knagende, schade verwekkende wormen of insecten in de vrije natuur. Hoewel de aard van die zogezegde wormen nooit duidelijk was, werden er wel allerhande namen aan gegeven. Soms werden ze genoemd naar de letsels die ze verwekten. Zo is ringworm (dermatofytose) in het Engels nog steeds een courante term. De meeste namen werden afgeleid van de locatie: haarworm, tandworm, navelworm, vingerworm. Een speciaal geval was de 'worm in de staart' waaraan allerhande ziekten werden toegeschreven. Denk aan 'het venijn zit in de staart' (in cauda venenum), waar men vermine verwart of mengt met venijn (gif). Ook in het West-Vlaams gebeurt dat: 't fernin even goed als 't fenin. Om daaraan te verhelpen maakte men een sneetje in de staart of sneed men de tip af. De ziekteverwekker zou met het uitdruppende bloed verdwijnen.

Naar Olbrechts, F. M. (1959). Over volkswetenschap in het algemeen en volksgeneeskunde in het bijzonder. *Volkskunde 60*, p. 133-179; Wouters, J. (1966). *Volksdiergeneeskunde*, Wetteren.

Luc Devriese

Trypanosoma equiperdum in the horse – a neglected threat?

Trypanozoma equiperdum *bij het paard – een onbekende bedreiging?*

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ABSTRACT

Dourine is a contagious disease caused by *Trypanosoma equiperdum* that is transmitted directly from animal to animal during coitus. Dourine is known as an important disease in many countries, and it threatens equidae worldwide. It is reported to be widespread in South America, Eastern Europe, Russia, Mongolia, Namibia and Ethiopia. The disease can be carried to various parts of the world through the transportation of infected animals and semen. Since knowledge of the prepatent infectiousness of a recently infected animal is lacking, introduction of the disease is in principle an ever-present threat. Definitive diagnosis depends on the identification of the parasite by means of direct microscopy. This is rarely possible in practice and therefore, diagnosis in the field is based on the observation of typical clinical signs, together with serological tests. This paper is an endeavour to review briefly and compile information on the appearance and importance of Dourine in terms of its epidemiological and clinical features, as well as on its diagnosis, treatment and prognosis.

SAMENVATTING

Dourine is een infectieuze en venerisch overdraagbare ziekte bij paarden die veroorzaakt wordt door *Trypanosoma equiperdum*. Deze aandoening is endemisch in Zuid-Amerika, Mongolië, Namibië, Ethiopië, Rusland en Oost-Europa en heeft een negatieve invloed op de gezondheid van de betreffende paardenpopulaties.

De ziekteverspreiding kan bespoedigd worden door het transport van geïnfecteerde dieren en sperma. De huidige kennis over de infectiviteit van het agens tijdens de prepatentperiode bij besmette dieren is minimaal. Aldus kan Dourine in landen verspreid worden die voorheen vrij waren van de ziekte. De eigenlijke diagnose is gebaseerd op de identificatie van de parasiet. Gezien dit in de praktijk meestal niet haalbaar is, wordt het klinisch beeld samen met niet-specifieke serologische testen gebruikt om de ziekte vast te stellen. In dit artikel wordt een overzicht gegeven over het belang van Dourine bij het paard. Verder worden de epidemiologie, het klinische ziektebeeld alsook de mogelijkheden van diagnostiek en behandeling besproken.

INTRODUCTION

Trypanosomosis is a parasitic disease caused by different species of flagellated protozoa belonging to the genus Trypanosoma, which inhabit the blood and various body tissues and fluids of vertebrate hosts. Non-Tsetse Transmitted Animal Trypanosomoses (NTTAT) result from infection by *Trypanosoma* (*T). vivax, T. evansi* and *T. equiperdum* (Touratier, 2000). However, the extent of tissue invasion varies among the different parasite species (Igbokwe, 1996; Radostitis et al., 1996). The disease is frequently fatal and is a serious constraint to agricultural production in large parts of sub-Saharan Africa, exhibiting direct impact on live-

stock productivity, livestock management and human settlement, and indirect impact on crop agriculture (Swallow, 2000).

Trypanosoma (T.) equiperdum differs substantially from other trypanosome species that are transmitted by invertebrate vectors. *T. equiperdum* is transmitted venereally directly from one infected horse to another. The trypanosomes, which are present in the seminal fluid and mucous membranes of the genitalia of the donor animal, are transferred to the recipient during sexual intercourse. Moreover, *T. equiperdum* further differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood (Barrowman 1976; Vulpiani et al., 2013; OIE, 2013).

Dourine caused by *T. equiperdum* is characterized mainly by fever, edematous swelling of the genitalia, cutaneous plaques and eruptions and neurological signs including incoordination, paralysis, ocular lesions (such as conjunctivitis, keratitis and mild corneal opacity), anemia and progressive emaciation (Barrowman 1976; Luckins 1994, Vulpiani et al., 2013). Clinical signs related to Dourine have been documented to be often obvious, but final diagnosis requires demonstration of the parasite through serological and molecular tests (OIE, 2013).

Among the various species of Trypanosoma, *T. equiperdum* and *T. evansi* infection in equines cause similar signs. In view of this fact, the actual course of the disease caused by these two trypanosome species was uncertain due to the close similarity in their ultrastructure, genetic makeup and antigenic nature, as has been demonstrated by the fact that the two species have shown similar genetic and antigenic expression (Touatier, 2000; Verloo et al., 2001; Claes et al., 2003a,b). However, recent findings of whole genome analysis of *T. evansi* and *T. equiperdum* provided new insights of their distinction and their relation with the different *T. brucei* subspecies (Birhanu et al., 2016; Cuypers et al., 2017). Both are evolved from *T. brucei* but with different geographical origins. The *T. evansi* genomes are related to the *T. brucei* genomes from Western Africa, whereas the *T. equiperdum* genomes are related to the *T. brucei* genomes from Eastern Africa (Carnes et al., 2015; Cuypers et al., 2017).

Currently, there are fragmented reports and findings about the disease in its occurrence, clinical signs, efficacy of treatment, etc. Therefore, the objective of this paper is to review and compile information on the appearance and importance of Dourine in terms of its epidemiological and clinical features, as well as on its diagnosis, treatment and prognosis.

APPEARANCE AND IMPORTANCE OF DOURINE

Dourine is known in most countries of the world as a notifiable disease (OIE, 2013) and it threatens equidae around the globe. The disease is reported to be widespread in South America (Samper and Tibary, 2006; Sellon and Long, 2007; Perrone et al., 2009; Sanchez et al., 2015), Mongolia (Clausen et al., 2003), Namibia (Kumba et al., 2002), Eastern Europe (Discontools, 2011) and Ethiopia (Alemu et al., 1997; Clausen et al., 1999; Fikru et al., 2010; Hagos et al., 2010a,b). Recently, the disease has also been observed in the south of Europe, in Italy (Scacchia et al., 2011; Pascucci et al., 2013).

The disease can be carried to various parts of the world through the transportation of infected stallions, mares, donkeys and semen. The spread of the causal agent by AI has not been confirmed; however, it could potentially occur since *T. equiperdum* is present in seminal fluid and genital tissues (Lelli et al., 2012). Besides venereal transmission, close contact between mare and foals, e.g. during nursing (Brun et al., 1998; Lelli et al., 2012), contaminated equipment, such as an artificial vagina and breeding phantom, or contaminated personnel can also cause transmission (Metcalf, 2001; Samper and Tibary, 2006).

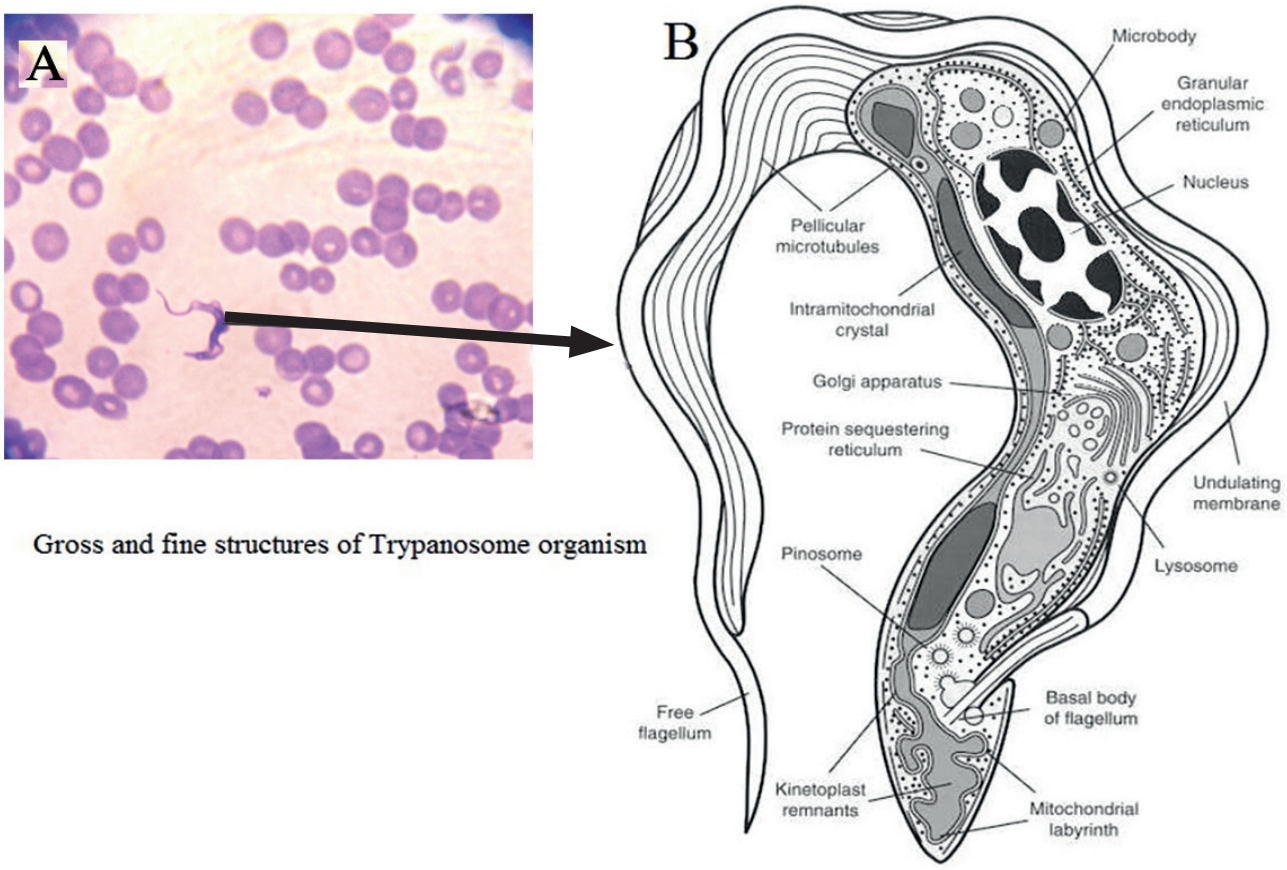
Today, despite the numerous benefits of shipping semen internationally, some serious threats remain unclear when the semen is contaminated with a communicable disease. Import regulations to prevent entry of the disease from endemic countries will require a negative Dourine test for horses and horse gametes. The current regulations vary between countries (IHSES, 2007; Calistri et al., 2013), which makes it difficult to comply with all of them when dealing for instance with cryopreserved semen. The import regulations vary from requiring only a permit for importation to several months of quarantine of the stallion. Due to the lack of knowledge of the prepatent infectiousness of the semen of recently infected animals, the transmission of disease through the transport of either the breeding stock or their gametes is a threat for the equine industry (Metcalf, 2001).

Although Dourine has a high mortality rate of up to 50%, some infected animals have been observed to recover spontaneously (Equimed, 2009; Ricketts et al., 2011; OIE, 2013). So far, there has been no known natural reservoir of the parasite other than infected equids. Donkeys and mules are more resistant than horses and may therefore remain unapparent carriers, in which the disease may often pass unperceived, even though their semen and vaginal secretions contain the infective trypanosomes (OIE, 2013).

ETIOLOGY

Trypanosomes are flagellated and elongated spindle-shaped protozoa with an average length of 20-30 µm and a width of 1.5 to 3.5 µm. The trypanosome cell has a blunted posterior end and a free flagellum at the anterior end (Matthews, 2005) (Figure 1).

The name *T. equiperdum* was postulated by Doflein in 1901 as cited in OIE (2013). *T. equiperdum* is a member of the non-tsetse-transmitted trypanosome group. The trypanosomes, which are strictly parasi-



Gross and fine structures of Trypanosome organism

tic, are flagellar protozoa that belong to the phylum of Sarcomastigophora, the order of Kinetoplastidae, the family of Trypanosomatidae and the genus of Trypanosoma, under the Salivarian group. The subgenus Trypanozoon includes the pathogenic species *T. evansi*, *T. brucei* and *T. equiperdum*. *T. brucei* is further divided into three subspecies: the animal pathogen *T. b. brucei* (ruminants, equines), and the two human pathogens responsible for human sleeping sickness, *T. b. gambiense* and *T. b. rhodesiense* (Hoare, 1972). *T. equiperdum* has recently come to be considered a subspecies of *T. brucei* based on molecular analysis (Claes et al., 2003b; Li et al., 2005, Lun et al., 2010; Schnaufer, 2010; Carnes et al., 2015; Wen et al., 2016).

Kinetoplastid flagellates contain their eponym kinetoplast DNA (kDNA), consisting of two types of interlocked circular DNA molecules: dozens of maxicircles and thousands of minicircles. The maxicircles have typical mitochondrial genes, most of which are translatable only after RNA editing. The minicircles encode guide RNA's, which are required for decrypting the maxicircle transcripts (Lai et al., 2008). *T. brucei* is a kinetoplastid trypanosome whereas *T. evansi* and *T. equiperdum* are dyskinetoplastic trypanosomes. *T. evansi* lack the maxicircle genes, but *T. equiperdum* does have, although with major deletions of some genes. So far, no akinetoplastic (lacking all kDNA) *T. equiperdum* have been observed (Birhanu et al., 2016). Two biological features differentiate *T. evansi* and *T. equiperdum* from the *T. brucei*. First, they do not use tsetse flies as the vector for transmission, since *T. evansi* is transmitted by biting flies and *T. equiperdum* by sexual contact (Desquesnes et al., 2013; Brun et al., 1998). Secondly, *T. evansi* and *T. equiperdum* are dyskinetoplastic, lacking part of or all (akinetoplastic) of their kDNA (Lai et al., 2008). Partial dyskinetoplastidy or total akinetoplastidy locks the trypanosome in the blood stream form trypamastigotes in the host, and transmission between vertebrates becomes purely mechanical, without further development in a vector (Bringuard et al., 2006).

Trypanosomes of subspecies *T. equiperdum* are rarely observed in the bloodstream of the host because they are normally localized in the capillaries of the mucous membranes of the urogenital tract (Brun et al., 1998). However, a few trypanosomes occasionally appear in the peripheral blood of animals (Brun et al., 1998; Fikru et al., 2010; Hagos et al., 2010c). The fact that foals have been found to be infected with *T. equiperdum* may indicate that the parasite can also be directly transmitted through udder lesions or milk, or during passage through the birth canal upon parturition (Schultz, 1935; Wang, 1988; Brun et al., 1998). Vertical transmission of *T. evansi* has already been reported in camels (Narnaware et al., 2016).

The true *T. equiperdum* strains successfully isolated and available for various studies are OVI, BoTat 1.1, Dodola 940 and 943 and the Mongolian isolates (IVM-t1) (OIE 2013; Birhanu et al., 2016, Suganuma et al., 2016).

CLINICAL SIGNS

Clinical symptoms, although not pathognomonic, can be of great help in diagnosing the disease in endemic areas. The presence of edematous swellings of the external genitalia, the development of plaques of the skin, and the appearance of nervous signs such as ataxia and incoordination are highly indicative for the disease (Vulpiani et al., 2013).

Substantial weight loss leading to severe emaciation, weakness and a generally poor condition has been reported in naturally infected as well as experimentally infected horses (Vulpiani et al., 2013). Hagos et al. (2010a, c) also reported marked muscular atrophy in the gluteal region, emaciation, weakness and poor body condition as important clinical signs in horses. However, the vulvar or preputial lesions of Dourine might be difficult to clinically differentiate from other diseases such as Equine Coital Exanthema (EHV3) in the early stages and in latent cases (Barrowman, 1976). Equine Herpes Virus (EHV3) infection is manifested by the appearance of vesicles, ulcers and depigmented spots on the vaginal mucous membrane (Blanchard et al., 1992; Studdert, 1996; Allen and Umphenour, 2004), which is similar to the genital form of Dourine. By contrast, however, EHV3 is self-limited and is not accompanied by emaciation, ataxia or incoordination.

Lesions in the genitalia are documented to be the first symptoms after *T. equiperdum* infection. In stallions, moderate edema of the scrotum and preputial sheath (Figures 2 E and F) is seen, accompanied by both preputial and urethral purulent discharge. In mares, edema of the vulva, accompanied by ulcers along the rim of the vulva, vaginal discharge mainly of the mucopurulent type with foul odour, and edema of the mammary gland and cloudy off-white mammary secretions have been documented as the prominent signs in the genital form of the disease. Later in the progression of the disease, edema of all ventral body parts, and cutaneous plaques all over the body can be seen. At this stage, the edematous plaques, especially on the genitalia, frequently ulcerate and become depigmented scars (Hagos et al., 2010a; Vulpiani et al., 2013) (Figure 2 D). This depigmentation has been indicated to be due to severe dermatitis with hydropic degeneration and necrosis of the keratinocytes and necrosis of basal cells including the melanocytes with excess free melanin pigment within the epidermis (Gizaw et al., 2017).

The next stage of Dourine is characterized by progressive anemia, with or without fever, and nervous disorders, mainly manifested as paraplegia and paralysis of the hind limbs, showing severe muscle atrophy and emaciation before death (Hoare, 1972; Stephen, 1986) (Figures 2 A, B and C).

Nervous signs such as stiffness, weakness, lameness in one or more hind legs, staggering, lack of coordination, inability to stand upright after prolonged sternal or lateral recumbence, ataxia and facial paralysis have also been reported by Hagos et al. (2010a) and Vulpiani et al. (2013).

DIAGNOSIS

Diagnosis has to be based on demonstrating the presence of the parasite itself or indirectly by antibody detection. However, there is no defined serological assay for T. *equiperdum* nor molecular markers

Figure 2. Symptomatology of Dourine infected horses. A. and B. Mare with weight loss and hind quarter paralysis. C. Stallion with hind quarter paralysis. D. Mare with depigmented scar at the vulva. E. and F. Swelling at the prepuce and scrotum from experimentally infected horses with *T. equiperdum* (mares are infected by artificial insemination after spiking the semen by *T. equiperdum* Dodola 943 and stallions are infected by the blood transfusion from these mares via intra venous route. Ethical clearance for this experiment was obtained from the Ethical Review Committee of Addis Ababa University College of Veterinary Medicine and Agriculture to use animals for experimentation (Ref. No: VM/ERC/004/07/015).

that can be used for clinical use so far. The clinical signs and gross lesions in diseased animals are suggestive, but cannot always be identified with certainty, especially in the early stages or in latent cases, and in cases of surra due to *T. evansi,* which exhibits similar clinical signs (OIE, 2013). Other conditions, such as equine coital exanthema or chronic irritation (e.g. urine scalding), might cause some similar clinical features (Blanchard et al., 1992; Studdert, 1996; Allen and Umphenour, 2004).

Differentiation between *T. equiperdum* and *T. evansi* based on parasite morphology is difficult in areas where both organisms are found (Brun et al., 1998; Sanchaz et al., 2015; Suganuma et al., 2016). So far, there are no defined serological assays for the true *T. equiperdum* and also no molecular markers for clinical use. Previously, Claes et al. (2004) and Fikru et al. (2010) used a PCR to amplify a portion of the RoTat 1.2. VSG coding gene in order to detect *T. equiperdum*. More recently, it has been shown that RoTat 1.2 VSG is not present on *T. equiperdum* (Birhanu et al., 2016), thus making previously reported findings equivocal.

Direct diagnosis: parasitological techniques

Wet, thick blood films can be examined microscopically (x400) under a coverslip. Detection of Trypanosomes moving in between the erythrocytes is a simple, although non-species specific, parasitological test. However, with a detection limit as high as 10,000 trypanosomes/ml, the technique has a very low sensitivity. Giemsa or stained thin blood films have a similarly low sensitivity. The sensitivity can be increased by applying concentration techniques such as used in the Woo test (Woo, 1970). This test includes a microcentrifugation of 50µl whole blood at 3000 g for 5 minutes. Subsequently, the capillary tubes are mounted in a special holder and the buffy coat is examined microscopically at magnification of ×100 to look for live parasites (Woo, 1970; Reid et al. 2001; Fikru et al., 2010). On microscopic evaluation, *T. equiperdum* and *T. evansi* cannot be differentiated from one another, a fact which makes the test less reliable in areas where both organisms are prevalent (OIE, 2012).

Immunohistochemistry and immunofluorescence

A more recent study managed to identify and localize trypanosome antigen directly in the tissue by immunochemistry, using Mayer's hematoxylin, in addition to an immunofluorescence staining, using diamidin phenylindole (DAPI) (Pasucci et al., 2013).

Animal inoculation

Mice, rats, rabbits and dogs are susceptible to laboratory adapted *T. equiperdum* strains (Brun et al., 1998; Claes et al., 2005; Akhmetova, et al. 2016). However, beside the animal welfare issues, it is often difficult to obtain a first passage from samples obtained from the host, and animal inoculation is of little use as a routine method of diagnosis.

Blood from suspected animals can be used as inoculants for laboratory rodents. Under laboratory conditions, dogs can develop Dourine. Different routes of infection, such as subcutaneous, intraperitoneal, intravenous, intraurethral and intravaginal transmission, were tested and all gave rise to clinical signs of Dourine. In dogs, inoculation of *T. equiperdum* produces the typical picture of Dourine with trypanosomes present in the lesions, but not in the blood, and the infection may last from one to several months (Stephen, 1986; Claes, 2003).

Strains of *T. equiperdum* were successfully isolated after intratesticular injection in the rabbit with blood or material from infected horses (Claes, 2003; Claes et al., 2005). Similarly, after the injection of udder secretions from clinically diseased mares into the scrotum of rabbits, *T. equiperdum* could be isolated successfully from the scrotal tissue homogenate of the rabbit. The new isolate from that rabbit could also be re-isolated from scrotal edema of another rabbit after successive inoculation, even after freezing of the inoculum in liquid nitrogen (Pascucci et al., 2013). However, blood and genital washes from serologically positive horses did not lead to infection when inoculated into mice and puppies (Alemu et al., 1997; Hagos et al., 2010a; Pascucci et al., 2013). Attempts to transmit the parasite to animals other than horses (rats by intra peritoneal way and dogs subcutaneously) were also unsuccessful with inocula of blood and Cerebro Spinal Fluid (CSF) known to contain living trypanosomes (Barrowman, 1976).

DNA-based technique

A highly sensitive method for detecting even a single parasite is the polymerase chain reaction (PCR) based on an amplification of trypanosomal DNA with a sensitivity of 0.5 pg of parasite DNA or one single parasite in 10µl blood (Wuyts et al., 1994). Using PCR of a 135 bp portion of a highly repeated region within the Trypanozoon subgenus and the OVI strain of *T. equiperdum* as positive control was used by Pascucci et al. (2013) to detect the organism from blood and other tissue fluids such as uterine and vaginal washings, cerebrospinal fluid, joint fluids, mammary secretion and urine. Moreover, solid tissues from udder, mammary and iliac lymph nodes, vulva, clitoris and uterus showed positive results for the parasite in Dourine affected animals.

Serology: Indirect diagnosis

From earlier studies, it was known that the diagnosis of *T. equiperdum* by standard parasitological techniques is difficult owing to the low numbers of parasites present in blood or tissue fluids and the frequent

absence of clinical signs of disease in the prepatent and the chronic phases. Consequently, the demonstration of trypanosomal antibodies in the serum has become the most important diagnostic technique in determining whether an animal is currently infected or has been previously in contact with the parasite (OIE, 2013). Serological testing by the complement fixation test (CFT) has been widely used in health certification of horses for export (Wassal et al., 1991).

Humoral antibodies are present in infected animals, whether they exhibit clinical signs or not. The CFT has been used to confirm clinical evidence and to detect latent infections (OIE, 2013). The reliability of CFT and IFAT for known *T. equiperdum* has been reported by Cauchard et al. (2014). However, uninfected equids, particularly donkeys and mules, often give inconsistent or nonspecific reactions because of the anti-complement effects of their sera. In this case, the indirect fluorescent antibody test (IFAT) is more adequate. Enzyme-linked immunosorbent assays (ELISAs) are also used (OIE, 2013).

However, the diagnostic antigens and antibodies currently available for use in sero-diagnostic tests are not specific for *T. equiperdum,* but react due to the cross-reactivity with the other Trypanosome spp. Significant improvements in Dourine sero-diagnosis will require the development of more specific *T. equiperdum*-subunit antigens. A recent publication reported the successful in vitro cultivation of *T. equiperdum* OVI parasites that can be used in complement fixation tests (Bassarak et al., 2016). This might be of great help in obtaining a specific diagnosis for *T. equiperdum*, since the OVI strain is one of the few genuine *T. equiperdum* strains generally available in reference laboratories (Claes et al., 2003b). Until all of these requirements have been achieved, however, the diagnosis of Dourine will of necessity involve the detailed history and the clinical and pathological findings, on top of the serology, to establish confirmation of the disease (Calistri et al., 2013).

Because of the lack of a specific antigenic marker to differentiate *T. equiperdum* from *T. evansi,* careful attention must be paid when choosing a strain to prepare such an antigen. According to recent data, true *T. equiperdum* needs to be differentiated from *T. evansi* (Claes et al., 2003a, b; 2004). The problem, however, is that many *T. equiperdum* strains have been found to be closely related to certain classes of *T. evansi* in cluster analysis by Random Amplified Polymorphic DNA (RAPD) and Multiplex-endonuclease genotyping (Claes et al., 2003b). With this in mind, the *T. equiperdum* Onderstepoort Veterinary Institute (OVI) and BoTat 1.1 strains have been found to be the most suitable for use as antigen sources. Single Nucleotide Polymorphism (SNP) within the F1-ATP synthase γ subunit gene provided an identifying characteristic of *T. evansi* as distinct from *T. equiperdum* without relying on VSG genes or kinetoplast DNA (Birhanu et al., 2016). A recent findings of whole genome SNP analysis of *T. evansi* and *T. equiperdum* also provided new insights in the origin of both species and their relation with the different *T. brucei* subspecies (Cuypers et al., 2017). This method may be the future key for differentiation of the two species and then developing specific markers for diagnosis.

Agglutination Test for Trypanosomosis

Card and Latex agglutination tests developed for *T. evansi* from RoTat 1.2 antigen have been implemented for Dourine diagnosis because of the cross-reactive nature of the antibodies of some strains of *T. equiperdum (*Claes 2002). Fikru et al. (2010) and Hagos et al. (2010 a,b) have used the test to diagnose Dourine in Ethiopia at the field level. However, there is no RoTat1.2 gene on true *T. equiperdum* strains (Birhanu, et al. 2016; OIE, 2013). Therefore, unless genuine antigens are identified from true strains of *T. equiperdum,* these agglutination tests will be no more functional to diagnose Dourine (OIE, 2013).

Indirect Fluorescent Antibody Test (IFAT)

The Indirect Fluorescent Antibody Test (IFAT) is frequently used in the diagnosis of Dourine as a confirmation test for a positive CFT result, since immunofluorescence is a more reliable and sensitive technique, though its interpretation is both subjective and labour intensive (Wiliamson et al., 1988). This test can be used in surveillance (prevalence of infection) and for the purpose of declaring a population free of the disease (OIE, 2013). This test has been used with success to diagnose Dourine in Italy (Pascucci et al., 2013).

Enzyme-Linked Immunosorbent Assay (ELISA)

Although the CFT has been used for many years to diagnose Dourine, it is considered to be less sensitive than ELISA and it has been suggested that ELISA could replace the CFT for animal health certification. The Enzyme-Linked Immunosorbent Assay (ELISA) is a very sensitive technique and its use for routine diagnostic serology of Dourine would provide a significant advantage over current serological tests if a defined antigen were to be used, since it would permit test standardization and more readily allow comparison of the test results among the different laboratories. In addition, ELISA testing lends itself to a far greater degree of automation, which makes it suitable for large numbers of samples (Wassal et al., 1991; Bishop et al., 1995).

Different authors have stated that the ELISA has a satisfactory concordance ratio with CFT and can be used to supplement CFT (Williamson et al., 1988; Alemu et al., 1997; Clausen et al., 2003). Similarly, Wasal et al. (1991) concluded that ELISA is a very sensitive test for Dourine compared to the CFT and IFA tests.

Recently, Davaasuren et al. (2017) have shown the use of ELISA to diagnose Dourine based on recombinant GM6 antigen (rTeGM6) derived from *T. equiperdum* isolated from the urethral mucosa of a clinically Dourine diseased stallion in Mongolia (Suganuma et al., 2016). The result showed a good diagnostic value in testing the sera of *T. equiperdum*-infected horses. However, it has been already shown to diagnose *T. evansi*-infected water buffalo, cattle, goats and sheep (Nguyen et al., 2015; Nguyen et al., 2014) by this method. This might be helpful in the diagnosis of non-tsetse transmitted horse trypanosomiases in the field, but the technique couldn't differentiate between the two species.

Genotype analysis is able to differentiate *T. evansi* from T. *equiperdum* (Carnes et al., 2015; Birhanu et al., 2016; Cuypers et al., 2017). This may lead to the development of other antigen-specific markers for *T equiperdum* in future ELISA.

TREATMENT

The World Organization for Animal Health (OIE, 2013) currently imposes the slaughtering of CFT-positive horses as an effective control strategy. In general, treatment may result in asymptomatic carrier animals and is as such not recommended in a Dourine-free territory because of fear for the continuing dissemination of the disease by the treated animals (Barrowman, 1976; Losos, 1986; OIE, 2000).

Evidence from in vitro drug sensitivity determination of *T. equiperdum* (Zhang et al., 1992; Brun and Lukins, 1994) indicates that Suramin, Diminazene, Quinapyramine and Cymelarsan are effective against trypanosome species. Hagos et al. (2010c) carried out *in vivo* efficacy testing of Diminazene diaceturate (Diminasan®) and bis (aminoethylthio) 4-melaminophenylarsine dihydrochloride (Cymelarsan®) on mice, which demonstrated that Cymelarsan® is effective, but that Diminasan®, on the other hand, fails even at high doses of up to 28 mg/kg body weight (four times the recommended dose in cattle) to cure any of the mice infected with the Dodola strain from Ethiopia.

Horses treated with Cymelarsan® at doses of 0.25 mg/kg and 0.5 mg/kg body weight showed no detectable parasitaemia 24 h after treatment. The mean PCV levels also improved after treatment, and seroreversion on card agglutination test for trypanosoma was observed starting from 150 and 170 days post treatment (Hagos et al., 2010c). This might have been due to the fact that the absence of the antigen source from the host system stopped the triggering of antibody production and consequently the antibodies were diluted in the serum. The study by Hagos et al. (2010c) showed improvement in body condition following the treatment of chronic infection and there was no relapse. Moreover, the clinical signs of incoordination of the hind legs, weakness and ventral oedema disappeared within 10 days, together with a progressive increase of the PCV. However, in recent reports, both Cymelarsan® and Diminasan® have been found to lead to relapse in treated mice (Habte et al., 2014), and parasites can still be found in the CSF of horses treated with Cymelarsan® (Cauchard et al., 2016).

Recently, ex vivo trypanocidal activity of 1-(2-hydroxybenzylidene) thiosemicarbazide against Venezuelan *T. equiperdum* strain has been reported (Parra et al., 2017). The compound exhibits a greater inhibitory activity of the parasite in the culture medium. In another recent report, in vitro laboratory tests of equine antimicrobial peptide (eCATH1) showed promising results relating to its trypanocidal activity on Trypanozoon spp through plasma membrane permeabilization and mitochondrial alteration. The administration of eCATH1 at a dose of 10 mg/kg to *T. equiperdum*-infected mice diminished the mortality rate. This finding suggests that eCATH1 can be considered as a candidate for the development of new therapeutic agents for the treatment of trypanosomosis (Cauchard et al., 2016).

PROGNOSIS

Horses treated with Cymelarsan showed elimination of the parasite from circulation within short periods of time (Hagos et al., 2010; Cauchard et al., 2016). However, since the parasite is a tissue parasite, it may hide in areas that cannot easily be reached by the drugs, thus resulting in relapse (Cauchard et al., 2016).

When horses are left untreated, the majority of cases will perish (Barrowman, 1976), or else they will develop a chronic form of Dourine with clinical signs as described above (Barrowman, 1976; Hagos et al., 2010a, c; Vulpiani et al., 2013). Parasitaemia may disappear after 80 days of infection even though the general body condition continues to progressively deteriorate (Barrowman, 1976; Hagos et al., 2010c). Infected animals became aparasitaemic after 80 days post infection, though they expressed parasitaemia again when challenged with immunosuppressive drugs (Hagos et al., 2010c). Therefore, it is assumed that the parasites can hide themselves from the immune system.

CONCLUSIONS

Dourine is a contagious disease that is caused by *T. equiperdum* and is transmitted directly from animal to animal during coitus. It is a notifiable disease in most countries and it threatens equidae worldwide. Since knowledge about the prepatent infectiousness of semen is lacking, introduction of the disease is in principle an ever-present threat. Clinical signs of the

disease are very similar to Surra caused by *T evansi*. Differentiation of *T. equiperdum* and *T. evansi* in areas where the two organisms coexist remains a challenge due to the absence of a specific antigen and molecular marker for clinical use. Characterization of the true *T. equiperdum* strains and the search for specific genes should be the focus of future research. The World Organization for Animal Health (OIE) imposes euthanasia of diseased animals due to the absence of an effective treatment and to prevent the dissemination of the disease. However, in vitro anti-trypanosomal activity of thiosemicarbazide compounds and equine antimicrobial peptide (eCATH1) seems to be a promising approach in the context of future treatment strategies.

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Spectrum of arthroscopic findings in 84 canine elbow joints diagnosed with medial compartment erosion

Artroscopische bevindingen bij 84 caniene ellebogen met erosie van het mediale gewrichtscompartiment

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ABSTRACT

Elbow dysplasia is an important cause of forelimb lameness in large breed dogs. The aim of this study was to report on the arthroscopic findings associated with medial compartment erosion (MCE) of the canine elbow joint. Retrospectively, records of 84 elbow joints from 66 dogs diagnosed arthroscopically with MCE were retrieved from a medical records database (2008 - 2012). The radiographic degree of osteoarthritis was determined. Arthroscopic images and videos were evaluated in detail. In nine joints (10.7%), MCE was the only pathological finding (= group 1). Group 2 (n = 50, 59.5%) consisted of elbows with MCE concomitant with medial coronoid process (MCP) pathology. In group 3 (n = 25 joints, 29.8%), MCE was diagnosed during a second-look arthroscopy in dogs presented with lameness after arthroscopic treatment for medial coronoid disease. There was a significant age difference ($p < 0.001$) between the groups, with dogs in group 1 being the oldest. Complete erosion of the medial compartment was most commonly found in group 1, whereas focal cartilage erosion was mostly identified in group 2. Overall, additional cartilage pathology of the lateral part of the humeral condyle and/or the radial head was recognized in 58.3% of the joints (49/84).

SAMENVATTING

Elleboogdysplasie is een veel voorkomende oorzaak van manken op de voorpoot bij grote hondenrassen. Het doel van deze studie was om de artroscopische bevindingen geassocieerd met erosie van het mediale elleboogcompartiment te beschrijven. Uit een medische database werden de dossiers geselecteerd van honden gediagnosticeerd met erosie van het mediale elleboogcompartiment. In de periode 2008-2012 werden 84 ellebogen (66 honden) gediagnosticeerd met deze aandoening. De radiografische graad van osteoartritis werd bepaald en de artroscopische beelden en video's werden in detail geëvalueerd. Bij negen gewrichten (10,7%) was erosie de enige pathologische bevinding (= groep 1). Groep 2 (n = 50, 59,5%) betrof ellebogen met erosie van het mediale compartiment en een letsel van de mediale processus coronoïdeus. In groep 3 (n = 25, 29,8%) werd erosie van het mediale compartiment gediagnosticeerd tijdens een revisie-artroscopie bij honden die reeds eerder behandeld werden voor een letsel van de mediale processus coronoïdeus. Bij het vergelijken van de groepen was er een significant verschil ($p < 0,001$) in leeftijd waarbij de honden in groep 1 het oudste waren. In groep 1 werd voornamelijk complete erosie van het mediale gewrichtscompartiment gezien, terwijl in groep 2 meestal focale erosie werd opgemerkt. Bij 58,3% van de gewrichten (49/84) werd eveneens kraakbeenschade in het laterale gewrichtscompartiment waargenomen.

INTRODUCTION

Elbow dysplasia is an important cause of forelimb lameness in large breed dogs (Fitzpatrick et al., 2009a; Michelsen, 2013; Barthélémy et al., 2014). The elbow dysplasia complex includes several pathologies, such as osteochondritis dissecans (OCD) of the medial part of the humeral condyle (MHC), an ununited anconeal process, joint incongruence and disease of the medial coronoid process, combined with a varying degree of

cartilage damage (Fitzpatrick et al., 2009; Michelsen, 2013; Barthélémy et al., 2014). Radioulnar incongruity is believed to be an important factor in the development of elbow dysplasia because of chronic supraphysiologic loading of the medial joint compartment (Gemill and Clements, 2007; Werner et al., 2009; Böttcher et al., 2013; Fitzpatrick et al., 2013). A histological study demonstrated fatigue microdamage in the region of the medial coronoid process (MCP) in dogs diagnosed with a fragmented MCP, supporting the hypothesis of chronic overload of the medial compartment in dysplastic elbows (Danielson et al., 2006). In a more advanced stage of medial coronoid disease, full thickness cartilage lesions with exposure of the subchondral bone (modified Outerbridge grade 4-5) (Fitzpatrick et al., 2009c, Vermote et al., 2010; Griffon, 2012; Coppieters et al., 2015) of the medial part of the humeral condyle (MHC) and the corresponding ulnar contact area develop (Fitzpatrick et al., 2009c, Vermote et al., 2010; Coppieters et al., 2015). This advanced cartilage damage can be referred to as medial compartment erosion (MCE) (Coppieters et al., 2015). It seems likely that fragmentation of the MCP and erosions of the medial compartment are attributable to a common pathway (Fitzpatrick et al., 2009a).

Clinical signs of MCE are similar to other lesions in the elbow dysplasia complex (Vermote et al., 2010; Coppieters et al., 2012; Griffon, 2012; Coppieters et al., 2015). However, most dogs with MCE demonstrate marked clinical abnormalities, such as obvious lameness, limited range-of-motion or signs of severe pain on elbow manipulation (Coppieters et al., 2015). Furthermore, little information is available on the type of dogs affected by MCE and the underlying pathology. Therefore, MCE is difficult to diagnose based on the history and clinical examination. Routinely used imaging techniques, such as radiography and computed tomography, cannot visualize articular cartilage (Moores et al., 2008; Coppieters et al., 2015). Arthroscopic inspection of the joint, the gold standard for articular cartilage evaluation (Van Ryssen et al., 1993; Meyer-Lindeberg et al., 2003), is the most reliable method to diagnose MCE. The modified Outerbridge classification (Griffon, 2012) is the best-known and most commonly used grading system to evaluate cartilage pathology based on the appearance of the articular surface and the depth of the lesions (Fitzpatrick et al., 2009b; Vermote et al., 2010; Griffon, 2012; Coppieters et al., 2015). Currently, the description of MCE is based on the modified Outerbridge classification grade of the MHC and the corresponding ulnar contact areas. Detailed information on the exact location or the extent of the lesions is lacking.

MCE is reported in three situations: As a single pathology, as a concomitant finding with MCP pathology and/or OCD of the MHC, or as a finding during second-look arthroscopy in dogs with persistent or recurrent lameness after initial arthroscopic treatment of medial coronoid disease (Vermote et al., 2010; Coppieters et al., 2012; Perry and Li, 2014; Coppieters et al., 2015; Coppieters et al., 2016a). The prevalence of those three groups in joints with MCE has not been reported yet.

MCE can be treated by load-transferring techniques or unicompartmental arthroplasty systems (Fitzpatrick et al., 2009c; Gutbrod and Guerrero, 2012; Smith et al., 2013; Franklin et al., 2014; Wendelburg and Beale, 2014). The extent of the cartilage damage within the medial joint compartment, for example cartilage damage expanding into the ulnar trochlear notch, might influence prognosis after treatment. In addition, these techniques require healthy cartilage of the lateral joint compartment. Although it has been reported that pathology of the lateral compartment is less commonly observed (Fitzpatrick et al., 2009c), the prevalence in joints with MCE has not been established yet. Therefore, it is difficult to determine the number of dogs that would benefit from those treatment options.

The aim of this retrospective study was to report on the arthroscopic lesions in dogs with MCE, determine the associated radiographic degree of osteoarthritis, describe the prevalence of the three groups in joints with MCE, and report on the prevalence of concomitant lesions of the lateral compartment. This knowledge may facilitate the diagnosis and treatment decision-making in the future.

MATERIALS AND METHODS

In this retrospective study, medical records of dogs that underwent elbow arthroscopy at the Department of Medical Imaging of the Faculty of Veterinary Medicine (UGhent) , from 2008 to 2012, were reviewed. To be included in this study, the following inclusion criterion had to be met: 1. clear elbow lameness and arthroscopic diagnosis of MCE (modified Outerbridge grade 4-5 of the MCP and MHC) (Table 1); 2. complete information about signalment, history, clinical and orthopedic examination; 3. a complete set of diagnostic high quality radiographs made one day to three weeks prior to the arthroscopic joint inspection; 4. still and video images of the arthroscopic inspection of the joint when diagnosed with MCE; and 5. still and video images of the first arthroscopy in patients diagnosed with MCE during a second-look arthroscopic procedure. Data collected from the medical records included gender, age at diagnosis, breed, duration of lameness, body weight, and in some cases, time between the first and second arthroscopy. In the case of dogs re-presented with lameness after the arthroscopic treatment of medial coronoid disease, the duration of lameness between the first and second arthroscopic treatment was considered. Twenty-two elbows diagnosed with MCE via arthroscopy were excluded because the inclusion criteria were not met. In

Table 1. Modified Outerbridge classification: grading system used to score the depth of the articular cartilage damage (Vermote et al., 2010; Griffon, 2012; Coppieters et al., 2015).

Modified Outerbridge classification	Description of gross cartilage findings
0	Normal cartilage
1	Chondromalacia (cartilage with softening and swelling)
2	Fibrillation Superficial erosions with pitting or a 'cobblestone' appearance Lesions that do not reach subchondral bone
3	Deep ulceration that does not reach the subchondral bone
4	Full thickness cartilage loss with exposure of the subchondral bone
5	Eburnated bone

case of diagnosis of bilateral MCE, both elbow joints were included in the study.

Radiographic examination of the elbows included an extended and flexed mediolateral view and a craniocaudal (15°-pronation) view. Radiographs were evaluated to determine the degree of osteoarthritis: 0 = no osteophytes, 1 = osteophytes ≤ 2 mm, 2 = osteophytes 2-5 mm and 3 = osteophytes > 5 mm (Hazewinkel, 2008).

Arthroscopy was performed with a 2.4 or 1.9 mm, 25° fore-oblique arthroscope (Richard Wolf, Knittlingen, Germany) using a standard medial approach (Van Ryssen et al., 1993). Digital still and video images were taken of all elbow joints. The arthroscopic findings were assessed by the first author and an experienced orthopedic surgeon at the same moment, until a consensus was reached. In patients with MCE, the extent of the cartilage lesions of the MHC and the MCP was evaluated and scored as focal, diffuse or complete erosion (Fitzpatrick et al., 2015; Coppieters et al., 2016b) (Figure 1). Radioulnar incongruity of the joint was evaluated, as previously reported by Wagner et al. (2007), at the base, midbody and apex of the MCP. Joints were classified as normal (no step), or mildly (step < 2 mm) or severely (step ≥ 2 mm) incongruent. Pathology of the lateral joint compartment, including the radial head and the lateral part of the humeral condyle, was also recorded. Additionally,

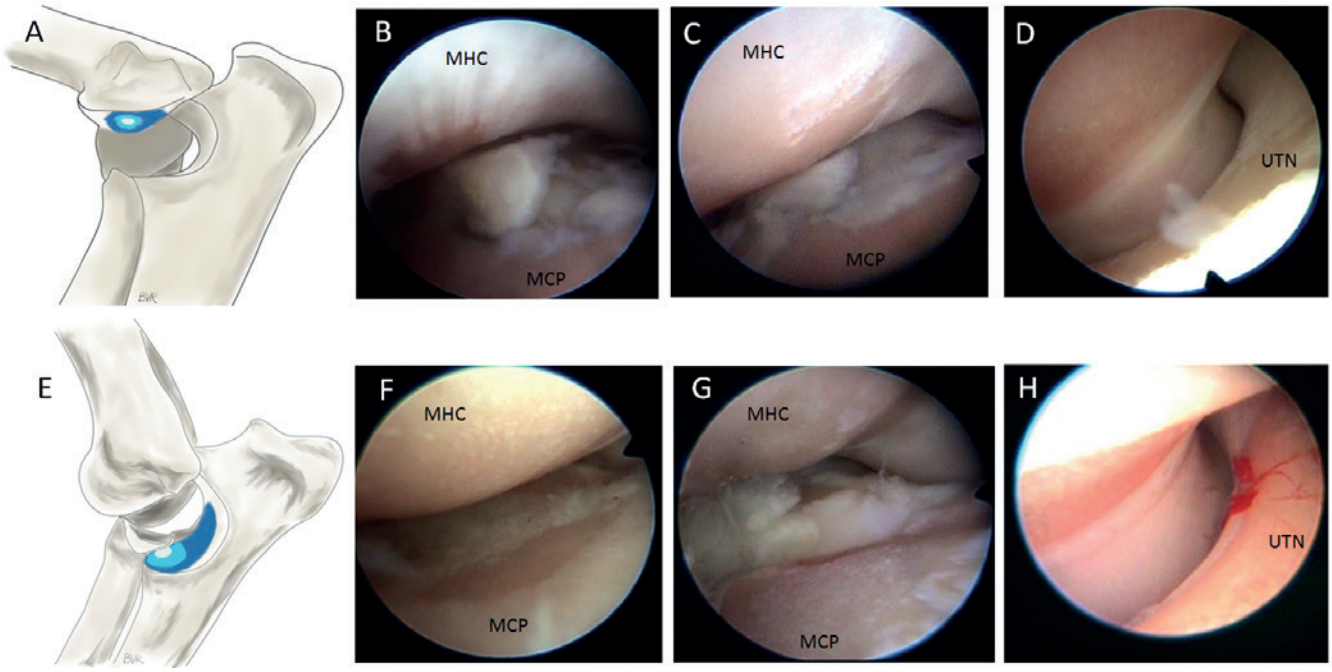

Figure 1. Schematic representation of the extent of the cartilage erosion on the medial part of the humeral condyle (MHC) (A) and the medial coronoid process (MCP) (E), and corresponding arthroscopic images, obtained via a standard medial approach (15), illustrating focal (B and F), diffuse (C and G) and complete (D and H) erosion of the MHC (top row) and the MCP (lower row) in elbow joints with medial compartment erosion. A, E. Focal erosions are very localized full-thickness cartilage erosions (white area). Diffuse erosions affect the majority of the cartilage surface of the MCP or the MHC but the cartilage of the trochlear notch or the corresponding humeral cartilage is still intact (light blue area). Complete erosions affect the entire surface of the MHC or the MCP including the cartilage of the ulnar trochlear notch (UTN) and the corresponding humeral cartilage (dark blue area).

Table 2. Breed distribution of 66 dogs with medial compartment erosion.

Breed	Number of dogs
Labrador retriever	27 (40.9%)
Cross-breed	7 (10.6%)
German shepherd dog	5 (7.6%)
Rottweiler	5 (7.6%)
Bernese mountain dog	5 (7.6%)
American bulldog	2 (3%)
Large Münsterländer	2 (3%)
Single breeds*	13 (19.7%)
Total	66 (100%)

*One of each: Airdale terrier, Belgian Malinois, Border collie, Chow Chow, Dutch patridge dog, French bulldog, Golden retriever, Greater Swiss mountain dog, Scotch collie, Sheltie, Tibetan mastiff, Weimaraner and White Swiss shepherd dog.

the presence of fissuring or fragmentation of the MCP and/or OCD of the MHC was recorded. After the retrospective evaluation of the radiographs and the data obtained by arthroscopy, the elbow joints were divided in three groups: group 1 = MCE as a single pathology, group 2 = MCE concomitant with a fragmented/fissured MCP and/or OCD and group 3 = MCE at second-look arthroscopy in dogs with persistent or recurrent lameness after arthroscopic treatment of medial coronoid disease (Vermote et al., 2010; Coppieters et al., 2013; Perry and Li, 2014; Coppieters et al., 2015, Coppieters et al., 2016a). For joints to be included in group 1, the absence of a concomitant fragment or fissure of the MCP needed to be confirmed by computed tomographic examination performed prior to the arthroscopy. The initial arthroscopic treatment performed in the joints of group 3 consisted of the removal of the fragmented or fissured part of the MCP and debridement of the remaining part of the MCP. All dogs in group 3 were clearly lame according to the owner and the veterinarian at the time of the second-look arthroscopy.

Postoperative care

The dogs were released on the day of the arthroscopic treatment. The dogs received a non-steroidal anti-inflammatory drug for three weeks. For all dogs, restricted exercise with leash walks was advised for a period of six weeks postoperatively.

Statistical analysis

Age and duration of lameness were compared for the three groups by the non-parametrical Kruskall-Wallis test. Post-hoc comparisons were done using the Wilcoxon rank sum test with the Bonferroni- correction. A Fisher-exact test with Monte Carlo simulation (2000 replicates) was performed to examine the relation between group and degree of osteoarthritis, incongruity, extent of MCP and MHC lesions, presence of linear abrasion tracks, presence of white areas and damage of the lateral compartment. The results are reported as percentages, median and range when appropriate. Statistical analysis was conducted with R (version 3.1.2, "Pumpkin Helmet") (R Core team, 2012). Significance was set at $P < 0.05$.

RESULTS

In the period from 2008 to 2012, a total of 587 elbows were diagnosed with lesion(s) included in the elbow dysplasia complex. The prevalence of MCE, diagnosed via arthroscopy, in this study population was 18.1% (n = 106, including cases with incomplete medical records). Of those 106 joints, 84 joints (66 dogs) were diagnosed with MCE and met the inclusion criteria for this study. Twenty breeds were represented, of which the Labrador retriever (27 dogs, 40.9%) was most common (Table 2). Male:female ratio was 1.6:1. Left and right distribution of MCE was almost equal (left: 47.6%, 40 elbows and right: 52.4%, 44 elbows) and 18 dogs had bilateral MCE. The median weight of the dogs was 32.5 kg (range 15 - 60kg). Nine joints (10.7%, 8 dogs) were assigned to group 1, 50 joints (59.5%, 40 dogs) to group 2 and 25 joints (29.8%, 19 dogs) to group 3. One dog was included in both groups 1 and 2 because of a bilateral diagnosis with MCE, which was considered a single pathology in one elbow and concomitant in the other elbow. Overall, a significant age difference ($p < 0.001$) between the groups was observed: dogs in Group 1 were significantly older than dogs in group 2 ($p < 0.001$) and dogs in group 3 ($p = 0.025$). Dogs in

Table 3. Distribution of degree of radioulnar incongruity per group of medial compartment erosion (MCE). Group 1 = MCE as a single pathology, group 2 = MCE concomitant to a coronoid pathology and group 3 = MCE at second-look arthroscopy in dogs with lameness after arthroscopic treatment of medial coronoid disease. Joint incongruity was evaluated during arthroscopic inspection and graded as normal (no radio-ulnar step), or mildly (step < 2 mm) or severely (step ≥ 2 mm) incongruent (Wagner et al., 2007).

	Total amount of joints	Congruent joints	Mildly incongruent joints	Severely incongruent joints
Group 1	9	9 (100%)	0 (0%)	0 (0%)
Group 2	50	13 (26.0%)	24 (48.0%)	13 (26.0%)
Group 3	25	14 (56.0%)	8 (32.0%)	3 (12.0%)

group 2 were significantly younger than dogs in group 3 ($p < 0.001$). The median age at diagnosis in group 1 was 8.5 years (range 6.6 - 11 years), in group 2 1.8 years (range 0.5 - 10.5 years) and in group 3 6.9 years (range 0.9 - 9.9 years). The median duration of lameness at the diagnosis of MCE was five months (range 1 – 78 months). No significant difference ($p = 0.199$) in duration of lameness was found between the three groups.

The radiographic degree of osteoarthritis per group is demonstrated in Figure 2. No significant difference ($p = 0.519$) was found between the three groups. In general, a high degree of osteoarthritis (degree 2 or 3) was present in 82.1% of the joints (69/84), 15.5% of the joints (13/84) only demonstrated small osteophytes (degree 1), and in 2.4% of the joints (2/84) no osteophytes were present (degree 0). In the 2 joints without osteophytes, some sclerosis of the ulnar trochlear notch was noticed.

A significant difference ($p < 0.001$) in degree of incongruity between the three groups was observed (Table 3). The distribution of focal, diffuse and complete pathology of the MHC and the MCP per group is shown in Tables 4 and 5. There was a significant difference in the extent of the cartilage erosion of the MCP ($p < 0.001$) and the MHC ($p < 0.001$) between groups. Focal erosion was more frequently seen in group 2, while complete erosion was predominantly seen in group 1.

All joints in group 1 were considered to be congruent on arthroscopic inspection (Table 3). The radio-

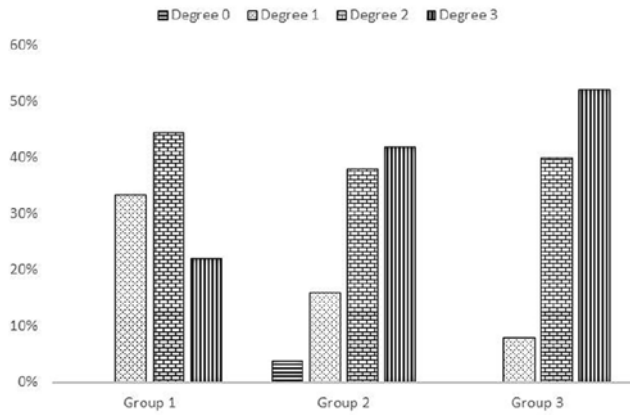

Figure 2. Distribution of the radiographic degree of osteoarthritis per group of medial compartment erosion (MCE). Group 1 = MCE as a single pathology, group 2 = MCE concomitant to a coronoid pathology and group 3 = MCE at second-look arthroscopy in dogs with lameness after arthroscopic treatment of medial coronoid disease. Osteoarthritis was scored as 0 = no osteophytes, 1 = osteophytes ≤ 2 mm, 2 = osteophytes 2-5 mm and 3 = osteophytes > 5 mm (Hazewinkel, 2008).

ulnar congruity of these joints was confirmed by the CT examination in all but one joint.

In group 2, MCE was diagnosed concomitant to various MCP lesions: a displaced fragment of the MCP in 42 joints (84.0%), a non-displaced fragment in 7 joints (14.0%) and a fissure in 1 joint (2.0%). In addition, a loose cartilage flap (OCD) of the MHC was

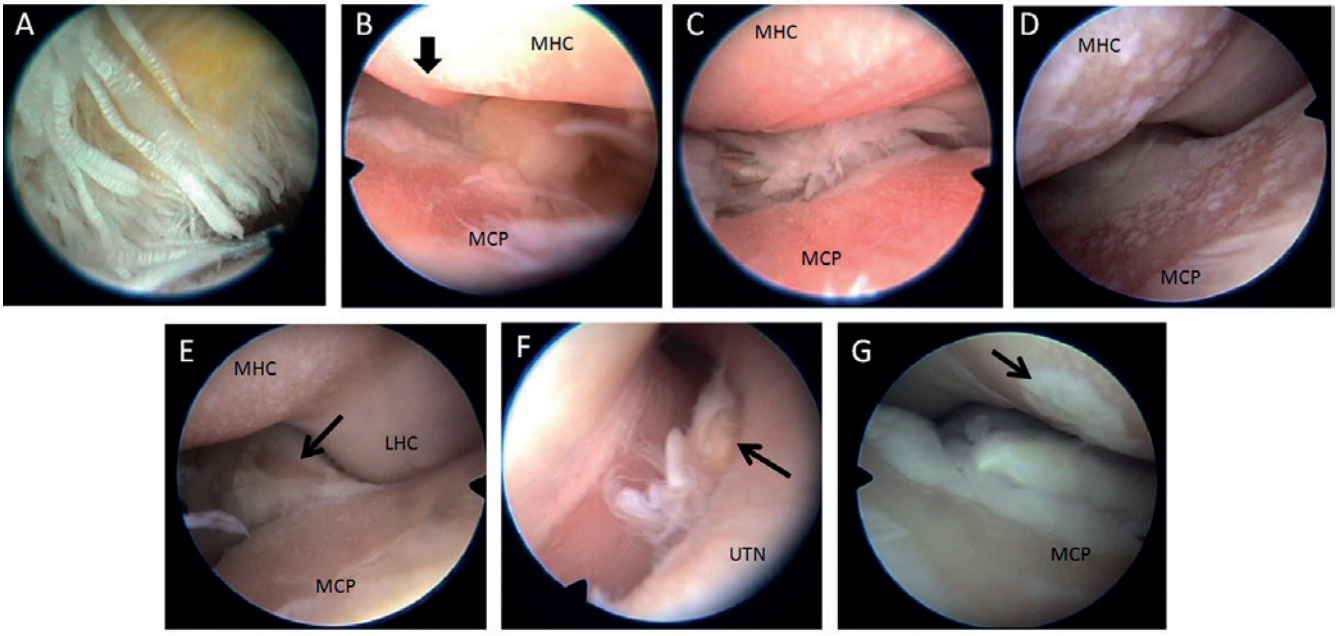

Figure 3. Arthroscopic images, obtained via a medial approach (Van Ryssen et al., 1993) of findings in dogs with erosion of the medial elbow compartment (MCP: medial coronoid process, MHC: medial part of the humeral condyle, UTN: ulnar trochlear notch). A. Fibrillated enthesis and yellow discoloration of the flexor muscles. B. A profound linear abrasion track in the subchondral bone of the MHC (arrow). C. Superficial linear abrasion tracks on the MHC. D. White areas in the eroded medial joint compartment. E. Full-thickness cartilage lesion of the medial part of the radial head (arrow). F. Cartilage damage of the lateral part of the humeral condyle (LHC) concomitant to the presence of an osteochondral fragment of unknown origin (arrow). G. OCD-like lesion (arrow) on the MHC surrounded by an area of full-thickness cartilage loss.

Table 4. Distribution of the extent of the cartilage pathology of the medial coronoid process (MCP) in joints with medial compartment erosion (MCE). Group 1 = MCE as a single pathology, group 2 = MCE concomitant to a coronoid pathology and group 3 = MCE at second-look arthroscopy in dogs with lameness after arthroscopic treatment of medial coronoid disease.

MCP	Focal erosion	Diffuse erosion	Complete erosion
Group 1	0 (0%)	1 (11.1%)	8 (88.9%)
Group 2	30 (60.0%)	13 (26.0%)	7 (14.0%)
Group 3	7 (28.0%)	8 (32.0%)	10 (40.0%)

Table 5. Distribution of the extent of the cartilage pathology of the medial part of the humeral condyle (MHC) in joints with medial compartment erosion (MCE). Group 1 = MCE as a single pathology, group 2 = MCE concomitant to a coronoid pathology and group 3 = MCE at second-look arthroscopy in dogs with lameness after arthroscopic treatment of medial coronoid disease.

MHC	Focal erosion	Diffuse erosion	Complete erosion
Group 1	0 (0%)	0 (0%)	9 (100%)
Group 2	22 (44.0%)	20 (40.0%)	8 (16.0%)
Group 3	1 (4.0%)	8 (32.0%)	16 (64.0%)

observed in 10 joints and an OCD-like lesion, consisting of fibrocartilaginous tissue attached in a rounded defect on the MHC, was noticed in 2 joints (Figure 3). Both OCD and OCD-like lesions were surrounded by an area of full-thickness cartilage loss. In 2 joints, an osteochondral fragment of unknown origin was found additional to an MCP lesion.

Group 3 consisted of 25 joints of dogs presented with lameness after arthroscopic treatment of medial coronoid disease, of which 11 joints (44.0%) demonstrated concomitant loose scar tissue, 5 joints (20.0%) attached scar tissue and 5 joints (20.0%) a calcified body at the level of the MCP. In the remaining 4 joints (16.0%), MCE was the only pathology found in the medial joint compartment. All these joints had been treated for disease of the MCP (chondromalacia (n = 4), fissure (n = 10), non-displaced fragment (n = 6), displaced fragment (n = 5)), of which 4 joints were treated for OCD of the MHC as well. During the first arthroscopy, 14 joints (56.0%) demonstrated normal cartilage, except for the MCP lesion or OCD of the MHC, 8 joints (32.0%) demonstrated mild cartilage damage (modified Outerbridge grade 1-2) and 3 joints (12.0%) already showed moderate cartilage damage (modified Outerbridge 3). The average time between the first and second arthroscopic inspection was 3.3 years (range 0.3 – 6.8 years). The median duration of lameness before the second-look arthroscopy was 5 months (range 2 – 78 months).

The occurrence of superficial and profound linear abrasion tracks in the subchondral bone of the MCP and the MHC is illustrated in Figure 4. In general, linear abrasion tracks in the subchondral bone were observed in joints with diffuse or complete erosions leading to bone-to-bone contact between the MHC and the MCP. No significant difference ($p = 0.073$) was found between groups in the presence of linear abrasion tracks on the MCP. However, a significant difference was found in the difference of linear abrasion tracks on the MHC ($p = 0.033$). Linear abrasion tracks on the MHC were most commonly found in group 1 (66.7% of the joints), and were least found in group 2 (24.0% of the joints). In 10 joints (11.9%), multiple white areas were noticed in the eroded part of the MCP and/or MHC (Figure 3). No significant difference ($p = 0.071$) was found between the groups in the presence of these areas. Additional cartilage pathology of the lateral joint compartment (radial head and/or lateral part of the humeral condyle) was recognized in 49 joints (58.3%). Both the radial head and the lateral part of the humeral condyle demonstrated cartilage damage in 14 joints (16.7%). The distribution and severity of pathology of the radial head and lateral part of the humeral condyle are illustrated in Figure 5. A significant difference ($p = 0.012$) was found between groups in the presence of damage of the lateral joint compartment. Some damage of the lateral compartment was identified in all joints in group 1, in 48% of the joints of group 2 and in 64% of the joints of group 3. In general, the only visible damage of the radial head was limited to its medial part (Figure 3). All joints with a complete erosion of the MHC still showed a sharp demarcation with the cartilage of the lateral joint compartment, even when some cartilage damage of the lateral part of the humeral condyle was observed.

Abnormalities at the enthesis of the flexor muscles, consisting of a fibrillated enthesis of the flexor muscles to the medial humeral epicondyle, a thickening of the flexor muscles or yellow discoloration of the flexor muscles, were detected in 25 joints (29.8%) (Figure 3).

DISCUSSION

MCE, an advanced stage of medial coronoid disease, has gained more interest and several specific treatment techniques have been developed to address this debilitating disease of the canine elbow joint. Joints with MCE can be divided into three groups: MCE as a single finding (group 1), MCE concomitant with a fragmented/fissured MCP and/or OCD of the MHC (group 2), and MCE as a finding during second-look arthroscopy in dogs with lameness after arthroscopic treatment of medial coronoid disease (Group 3) (Vermote et al., 2010; Coppieters et al., 2012; Perry and Li, 2014, Coppieters et al., 2015, Coppieters et al., 2016a). Limited information is available on the prevalence of the three specific groups of MCE and their characteristics. This study demonstrates significant differences in age, joint incongruity, extent of the cartilage lesions, damage in the lateral joint compartment and presence of linear abrasion tracks on the MHC between the three groups of MCE and illustrates that several additional pathologies may occur in these joints.

The high number of Labrador retrievers in this study is prominent. Other studies reporting on treatment techniques for MCE also mention a high frequency of Labrador retrievers (Fitzpatrick et al., 2009c; Fitzpatrick et al., 2015). Possibly, this breed is more vulnerable to cartilage degeneration. More likely, this high number of Labrador retrievers affected with MCE can be explained by the popularity and the high prevalence of elbow dysplasia in general within this breed (Meyer-Lindeberg et al., 2003; Fitzpatrick et al., 2009a; Coopman et al., 2014).

The dogs in group 1 were significantly older than dogs in the other two groups and had a minimum age of 6.6 years old. This finding is in contrast with other studies that reported MCE as a single finding in younger dogs as well (Olsson, 1987; Vermote et al., 2010). Possibly, subtle concomitant pathology of MCP or MHC may have been overlooked in these younger dogs. Next to the older age of the dogs, complete erosion of the medial compartment was most frequently seen in joints in group 1. This finding is consistent with the conclusion of a study on Labrador retrievers with disease of the MCP reporting a significant relationship between age and the global cartilage pathology (Farrell et al., 2014).

The radiographic degree of osteoarthritis was high in the majority of the joints. This could be expected since it is known that there is a correlation between the radiographic degree of osteoarthritis and articular cartilage damage in the medial elbow compartment (Fitzpatrick et al., 2009a; Vermote et al., 2010; Farrell et al., 2014). However, two joints diagnosed with MCE demonstrated no osteophytes. A similar finding has been reported by Vermote et al. (2010). Therefore, the diagnosis of MCE cannot be excluded based on radiographic examination only.

The etiology of MCE has not been clarified yet. A common pathway between disease of the MCP and MCE has been suggested (Fitzpatrick et al., 2009a). Radioulnar incongruity may generate supraphysiologic overload of the medial joint compartment (Gemill and Clements, 2007; Werner et al., 2009, Böttcher et al., 2013, Fitzpatrick et al., 2013). However, in the present study several joints with MCE were considered congruent based on arthroscopic joint inspection. Possibly, incongruity was only temporary present during skeletal growth (Michelsen, 2013), or joint congruity was misinterpreted during arthroscopy. Although arthroscopy has been described to be an appropriate technique for the diagnosis of radioulnar incongruity (Wagner et al., 2007; Werner et al., 2009), it is known that insertion of the arthroscope and/or the non-weight bearing position of the leg can influence the interpretation of joint incongruity (Skinner et al., 2015). Nevertheless, the remarkable finding of all joints in group 1 being congruent on arthroscopy could be confirmed by CT examination in all but one joint. Therefore, it is possible that other factors may be involved in the development of medial compartment erosion, especially when it is presented as a single finding.

In this study, MCE was most often identified concomitant with a MCP lesion (group 2). A displaced fragment of the MCP was the predominant concomitant finding in this group. Conflict between the intact portion of the MCP and the MHC and/or chronic inflammation created by the presence of a fragment might have caused the development of MCE (Samoy et al., 2012; Michelsen, 2013). Linear abrasion tracks in the subchondral bone were least found in this group. Those linear abrasion tracks, possibly caused by profound humeroulnar conflict, were mostly observed in joints with diffuse or complete erosions leading to bone-to-bone contact between the MHC and the MCP. Since focal erosion was most frequently observed in group 2, this could explain the lower occurrence of linear abrasion tracks in this group.

MCE in dogs with lameness after arthroscopic treatment of disease of the MCP was the second largest group in this study. It has been suggested that fragmentation of the MCP is a first step of medial coronoid disease before erosion of the more resilient part of the MCP occurs (Fitzpatrick et al., 2009a). Indeed, the first arthroscopic treatment of these joints only consisted of fragment removal, making further supraphysiologic loading of the remaining part of the MCP possible. However, more than half of the joints of group 3 were considered congruent joints. Although elbow congruency is challenging to assess, it is likely that other factors or other unknown underlying conditions are involved in the etiopathogenesis of these severe cartilage erosions. For example, it is possible that the initial surgical intervention, interrupting the joint surface, triggered the progression of degenerative cartilage damage (Coppieters et al., 2016a).

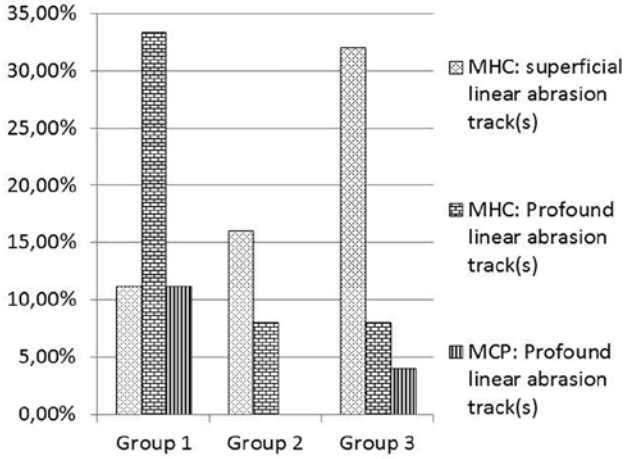

Figure 4. Prevalence of linear abrasion tracks in the subchondral bone of the medial part of the humeral condyle (MHC) and the medial coronoid process (MCP) in joints with medial compartment erosion (MCE). Group 1 = MCE as a single pathology, group 2 = MCE concomitant to a coronoid pathology and group 3 = MCE at second-look arthroscopy in dogs with lameness after arthroscopic treatment of medial coronoid disease.

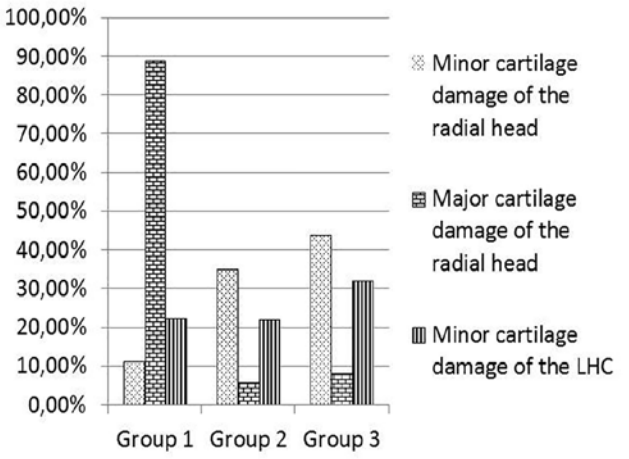

Figure 5. Prevalence of major (modified Outerbridge grade 4-5) and minor (modified Outerbridge grade 1-3) cartilage damage of the radial head and the lateral part of the humeral condyle (LHC) in joints with medial compartment erosion (MCE). Group 1 = MCE as a single pathology, group 2 = MCE concomitant to a coronoid pathology and group 3 = MCE at second-look arthroscopy in dogs with lameness after arthroscopic treatment of medial coronoid disease.

Information on the prevalence of pathology of the lateral elbow compartment in joints with MCE is scarce in the literature, since MCE only relates to cartilage damage of the MHC and the corresponding ulnar contact area (Fitzpatrick et al., 2009c, Vermote et al., 2010; Coppieters et al., 2015). In general, it is stated that pathology of the lateral compartment is less common (Fitzpatrick et al., 2009a). In this study, cartilage damage of the radial head and/or the lateral part of the humeral condyle has been identified in more than half of the joints with MCE, of which minor damage (modified Outerbridge grade 1-3) of the radial head was the most frequent finding. In vitro studies have demonstrated that the proximal articular surfaces of the radius and the ulna contribute almost equally to load transfer through the elbow joint (Preston et al., 2000; Mason et al., 2005). The radial contact area is located on the caudomedial aspect of the radial articular surface, with its longest dimension oriented mediolaterally (Preston et al., 2000). In the present study, damage of the radial articular surface was only observed in the medial part of this area (Figure 3). Possibly, only the part of the radial head that corresponds with the eroded MHC displays cartilage loss. Therefore, detailed inspection of the radial articular surface is recommended during arthroscopic joint inspection, and additional damage of the radial head should be taken into account before specific treatment methods for MCE, such as a unicompartmental elbow arthroplasty or a sliding humeral osteotomy, are considered (Fitzpatrick et al., 2009c; Franklin et al., 2014).

In ten joints (11.9%), multiple white areas were noticed within the eroded part of the MCP and/or MHC. These might be areas of fibrocartilaginous repair tissue. However, due to the retrospective nature of this study, histopathological examination of these lesions wasnot performed. Thus, it cannot be excluded that these areas are resilient parts of articular cartilage.

Abnormalities at the enthesis of the flexor muscles were detected in almost one third of the joints. It is known that arthroscopy often reveals abnormalities at the enthesis of the flexor muscles in joints with elbow dysplasia lesions (de Bakker et al., 2013). The clinical relevance of these findings is unclear.

In veterinary medicine, the modified Outerbridge classification system is considered the 'gold standard' for arthroscopic evaluation of cartilage lesions (Fitzpatrick et al., 2009a; Farrell et al., 2014). However, this grading system only takes into account the depth of the cartilage lesion, without considering the extent of the lesion. A recent study evaluated the global articular cartilage damage in Labrador retrievers using a composite cartilage score (CCS) which accounted for lesion depth, location and surface area (Farrell et al., 2014). However, this CCS system was not suitable for our study, since nearly all joints with modified Outerbridge grade 4 and 5 (MCE) would have been classified in the highest category being 'severe disease'. Therefore we proposed a new classification scheme, using the terms focal, diffuse and complete, to determine the extent of the cartilage lesions in joints with MCE. A limitation of this classification is that it is rather subjective and not quantitative, since our data were collected retrospectively. The use of the proposed grading scheme allows a classification that may be useful to decide on the type of therapeutic approach as well in the future.

In conclusion, this study provides an overview

of the arthroscopic findings in three types of MCE. Significant differences in age, radioulnar incongruity, extent of the cartilage lesions, damage in the lateral compartment and presence of linear abrasion tracks on the MHC were identified between the three groups. These findings may contribute to the determination of underlying pathophysiological characteristics that lead to MCE. In addition, this study illustrated several additional pathologies that can occur in joints with MCE, including cartilage damage of the lateral joint compartment, flexor enthesopathy and/or a fragment, a calcified body or loose scar tissue at the level of the MCP. These additional pathologies should not be overlooked when MCE is diagnosed and should be taken into account when treatment-decisions are made.

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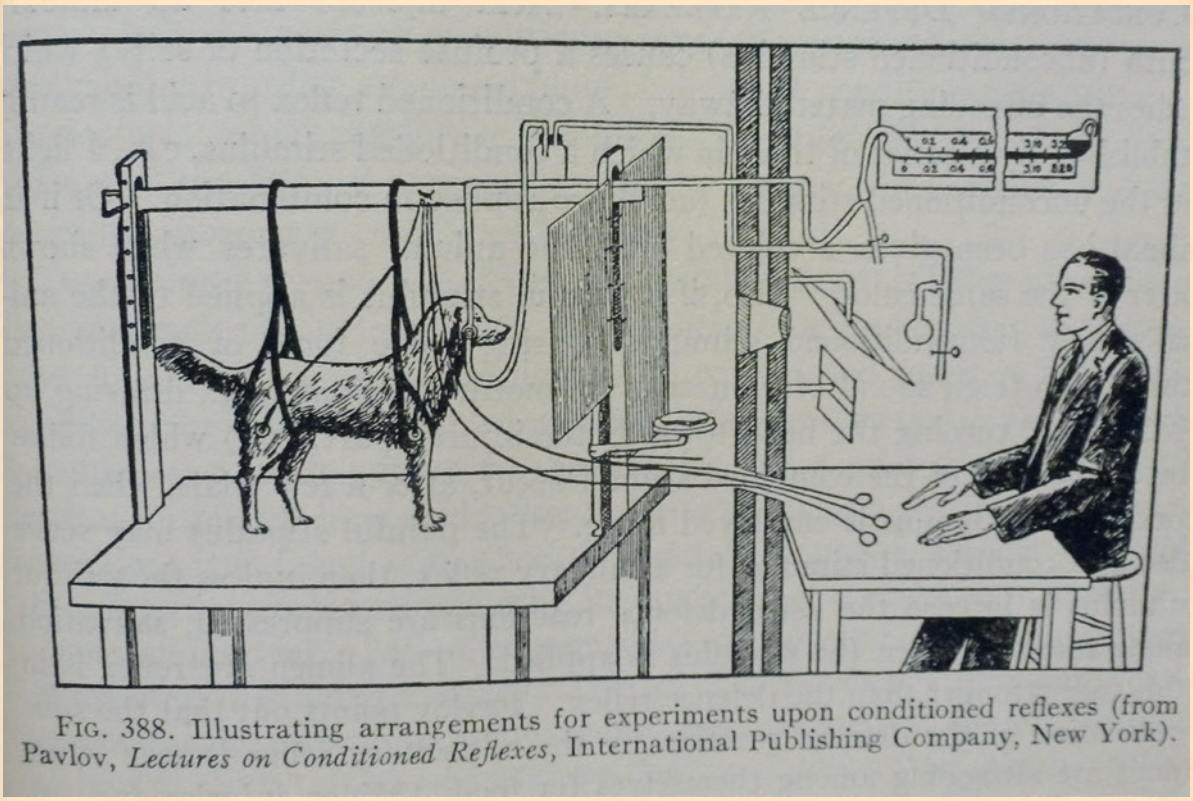

Fig. 388. Illustrating arrangements for experiments upon conditioned reflexes (from Pavlov, *Lectures on Conditioned Reflexes*, International Publishing Company, New York).

Intratumoral chemotherapy in an integumentary squamous cell carcinoma in a cockatiel *(Nymphicus hollandicus)*

Intratumorale chemotherapie van een plaveicelcarcinoom van de huid bij een valkparkiet (Nymphicus hollandicus)

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ABSTRACT

An eight-year-old, female cockatiel (*Nymphicus hollandicus*) was presented with anorexia, lethargy, a mass at the lower side of the wing and discoloration of the feathers. Physical examination showed an ulcerated nodular integumentary lesion of approximately 4 cm³ ventromedial on the wing at the side of the propatagium and the humerus. Lateral and ventrodorsal radiographs revealed only hepatomegaly. After a stabilization period, surgical excision of the tumor was performed. Based on histopathological evaluation and bacterial culture of the surgically removed tissue, the lesion was typed as an integumentary squamous cell carcinoma with secondary bacterial infection (*Corynebacterium sp*). Four weeks postoperative, the tumor had recurred. Chemotherapeutic treatment was started with intratumoral carboplatin (1.5 mg/cm³) once a week. Because of further tumor growth after the second administration of carboplatin, resection of the mass was performed before the next infiltration. However, the bird died during anesthesia. Despite increase in tumor size, histopathological and immunohistochemical evaluations showed degeneration of the tumor with intercellular edema and vacuolization of the tumor cells, presumably resulting from carboplatin administration. More research is needed to investigate the efficacy and safety of the intratumoral administration of carboplatin as a treatment option in birds with integumentary squamous cell carcinoma.

SAMENVATTING

Een acht jaar oude, vrouwelijke valkparkiet (*Nymphicus hollandicus*) werd aangeboden met klachten van anorexie, lethargie, een massa onderaan de vleugel en verkleuring van het vederkleed. Het lichamelijk onderzoek toonde een ulceratief nodulair letsel van de huid van ongeveer 4 cm³, ventromediaal op de vleugel ter hoogte van het propatagium en de humerus. Op een laterale en ventrodorsale radiografie was er als enige afwijking hepatomegalie zichtbaar.

Na een stabilisatieperiode werd er een chirurgische excisie van de tumor uitgevoerd. Uit het histopathologisch onderzoek en de bacteriële cultuur van de chirurgisch verwijderde massa bleek de laesie een integumentaal plaveicelcarcinoom te zijn, secundair bacterieel geïnfecteerd met *Corynebacterium sp*. Vier weken postoperatief was de tumor gerecidiveerd. Een behandeling met chemotherapie werd opgestart, waarbij éénmaal per week carboplatine intratumoraal (1,5 mg/cm³) werd toegediend. Omwille van de verdere tumorgroei na de tweede toediening van carboplatine werd de massa gereseceerd voordat de volgende toediening van carboplatine plaatsvond. De vogel overleed echter tijdens de anesthesie. Histopathologisch en immunohistochemisch onderzoek toonde degeneratie van de tumor met intercellulair oedeem en vacuolisatie van de tumorcellen aan, ondanks de toenemende tumorgrootte. Vermoedelijk was dit het resultaat van de carboplatinetoediening. Verder onderzoek is nodig om de werkzaamheid en veiligheid van de intratumorale toediening van carboplatine te bestuderen als behandelingsoptie bij vogels met een plaveicelcarcinoom van de huid.

INTRODUCTION

Life expectancy of companion birds is increasing due to improved husbandry, nutrition and veterinary care. Consequently, a growing number of degenerative and neoplastic diseases in birds are diagnosed and treated by veterinarians (Robat et al., 2017). Neoplastic processes are more frequently diagnosed in cage birds than in aviaries or in wild birds (Siegfried, 1982). Among psittaciformes, the budgerigar (*Melopsittacus undulatus*) is most frequently diagnosed with neoplasia (Ratcliff, 1933). A private exotic species pathology service showed that of the geriatric population, 66.7% of budgerigars submitted to the clinic showed neoplastic disease; in geriatric cockatiels (*Nymphicus hollandicus*) this was 49.6% while in other psitacciformes species only one third had a neoplastic process (Reavill and Dorrestein, 2010). Neoplastic processes of the skin are the most frequently diagnosed tumors in pet birds (31.7%), followed by the urinary system (25.1%) and the reproductive system (17.3%) (Leach, 1992).

A squamous cell carcinoma (SCC) is a malignant neoplasia, which originates from the squamous epithelium of the skin or the proximal part of the gastrointestinal tract (Koski, 2002; Reavill, 2004; Klaphake et al., 2006). A SCC is histologically characterized by infiltrative nests and cords of moderately undifferentiated to poorly differentiated squamous cells, which frequently form so called 'keratin pearls', presenting as central cores of compressed and laminated keratin (Reavill, 2004). Of the pet birds diagnosed with a neoplastic process, 1.7% suffer from SCC (Malka et al., 2005). The bird species that are most commonly diagnosed with SCC are the cockatiel, the amazon parrot (*Amazona spp*.) and the budgerigar (Koski, 2002; Reavill, 2004; Klaphake et al., 2006). The predilection sites of SCCs in birds are the distal part of the wing, phalanx and uropygial gland (Koski, 2002; Lightfoot, 2010).

Multiple risk factors have been associated with the development of integumentary SCC in humans and animals (Klaphake et al., 2006). It is known in mammalian species that long-term exposure to ultraviolet (UV) radiation of poorly pigmented zones is a risk factor for the development of SCC (Meleo, 1997; Reavill, 2004; Klaphake et al., 2006; Abu et al., 2009). However, in birds, SCC mostly occur in skin regions, which are not exposed for a long period of time to sunlight (Reavill, 2004). In addition, chronic exposure to cigarette smoke, chronic skin disease, chronic feather picking, a species-specific poxvirus in young chickens, and other unknown factors have been documented as predisposing factors for the development of integumentary SCC in birds (Koski, 2002; Reavill, 2004; Klaphake et al., 2006).

As in mammals, the prevalence of metastasis of SCC in birds is low (Koski, 2002; Reavill, 2004; Malka et al., 2005; Klaphake et al., 2006; Abu et al., 2009; Lightfoot, 2010). Metastasis of SCC in birds is mostly found in the lungs, the spleen, the cortex of bones and the liver (Abu et al., 2009). Although the tendency to metastasize is very low, the capacity of tumor invasion into the surrounding tissue is generally very high, which results in a guarded prognosis (Manucy et al., 1998; Koski, 2002; Reavill, 2004; Malka et al., 2005; Klaphake et al., 2006; Abu et al., 2009; Lightfoot, 2010). Additionally, severe necrosis caused by the fast growth of the tumor or related to treatment, especially chemotherapy and radiation, might worsen the prognosis because of an increased risk of developing septicemia following secondary infection (Klaphake et al. 2006; Lightfoot 2010). The prognosis is also negatively influenced by age and delayed diagnosis (Manucy et al., 1998; Klaphake et al., 2006).

The use of chemotherapeutics as a possible treatment strategy in avian oncology has been steadily increasing in recent years (Zehnder and Kent, 2011). Platinum-based antineoplastic agents are commonly used to treat integumentary neoplastic processes (Mo et al., 2014). However, defining the optimal treatment protocol for tumors in birds is still largely empirical. The treatment protocols used in birds have been extrapolated from other companion animals, such as dogs and cats or humans (Graham et al., 2004). Antonissen et al. (2015) demonstrated a correlation between body weight (BW) and carboplatin elimination half-life ($T_{1/2el}$) to allow allometric scaling of carboplatin in avian species. Cisplatin and carboplatin form reactive intracellular platinum complexes that bind to nucleophilic groups in the DNA, producing both inter- and intra-strand crosslinks that inhibit DNA replication, RNA transcription and protein synthesis, resulting in apoptosis (Mo et al., 2014). Carboplatin was developed to reduce the myelosuppression, nephrotoxicity and nausea associated with cisplatin, a first-generation platinum agent (Ito et al., 2013).

In this report, the first case of intratumoral administration of carboplatin in a SCC in a cockatiel is described.

CASE

An eight-year-old, female cockatiel was presented with anorexia, lethargy, discoloration of the feathers and a progressive, slow-growing mass at the ventral side of the left wing. The mass had been observed approximately two months before presentation. The owner observed that the tumor started bleeding after handling the bird. A few days prior to the moment of initial presentation, the bird showed polydipsia (PD). The bird was housed in an indoor cage together with another cockatiel and was fed a diet consisting primarily of seeds with additional bird-snacks and table scraps. Drinking water was supplemented with a mixture of vitamins (Multi-Vit, Beaphar, Poperinge, Belgium).

On physical examination, the bird showed a poor general and body condition and weighed 90 g. The skin was less densely feathered and the area of the left eye, ear and dorsal side of the wing were bald. In contrast to the original color of the feathers (white to light yellow), all feathers had been intense yellow since one month. An ulcerated nodular integumentary lesion of approximately 4 cm³ was observed, which was located ventromedial on the left wing at the side of the propatagium and the humerus (Figure 1). Cytological examination after a hemacolor staining of a fine-needle aspiration of the nodule revealed multiple epithelial cells with irregular size and several nucleoli per cell, which suggested a neoplastic process. Lateral and ventrodorsal radiographs revealed hepatomegaly, but no signs of metastases. Further diagnostic evaluation, including blood hematology and biochemistry, liver biopsy and PCR test for *Chlamydia psittaci* were declined by the owner because of financial constraints. In summary, anamnesis and clinical examination suggested an integumentary neoplasia and chronic liver failure. The differential diagnosis of the liver disease was septicemia, hypovitaminosis A, neoplasia and degenerative liver disease.

During a period of seven days, the bird was stabilized supporting liver function by oral administration of milk thistle (75 mg/kg BW, PO, BID, Elusanes Mariadistel, Pierre Fabre Santé Benelux, Belgium), which is a natural herb that has antioxidant and anti-inflammatory properties and B-chol, a mixture of sulphuric amino acids, biotin, sorbitol and vitamin B12, (1ml / 100 ml drinking water) (Oropharma, Belgium) to stimulate the excretion of bile. Besides, enrofloxacine (20 mg/kg BW, PO, BID, Baytril, Bayer Animal Health, Belgium) was administered, since the diagnostic tests could not exclude septicemia. Surgical intervention was performed to remove the tumor. Induction was achieved with 5% isoflurane in 1.5 L/min oxygen administered via mask. After induction, anesthesia was maintained with 2% isoflurane in 1.5 L/min oxygen. The mass was surgically removed at the base of the tumor and sutured with vicryl 4.0 (Ethicon, Sommerville, NJ, USA). Due to the location of the mass, it was impossible to include a surgical margin around the tumor. Wing amputation was declined by the owner. The mass was fixed in a 4%-neutral-buffered formalin solution and processed afterwards according to the conventional methods for hematoxylin and eosin (HE) staining. At the end of the surgery, a swab for bacterial culture was taken from the surgical field at the side where the base of the tumor was resected.

Histopathological evaluation of sections of the surgically removed tissue showed a vaguely defined mass consisting of strains and clumps of large, polygonal cells with clearly defined cytoplasm and large, round and pale nuclei with fine fibrous chromatine and one to two large, eosinophylic nucleoli. Furthermore, a moderate anisokaryosis and mild anisocytosis, and an occasional formation of syncytial cells were observed, intermixed with a moderate amount of fibrovascular stroma. At multiple sites, large pearls of keratin were observed (Figure 2). The tissue was mildly infiltrated with heterophils. The mitotic index was three mitotic figures per high-power field (HPF). Based on histopathological examination, the tumor was typed as an integumentary SCC. The superficial part of the tumor was ulcerated and infiltrated with bacteria. Bacterial culture of the wound revealed contamination with *Corynebacterium sp*., and based on the antimicrobial drug susceptibility profile, the postoperative treatment consisted of amoxicillin/clavulanic acid (125 mg/kg BW, PO, BID, Noroclav, Norbrook Laboratories Limited, Northern Ireland) during two weeks, B-chol and milk thistle.

Sixteen days postoperative, the general clinical condition of the bird was improving. The animal was more alert, showed increased preening behavior and the PD had disappeared. However, the surgical wound did not heal, suggesting either a bacterial infection or a reoccurrence of the SCC. Bacterial and mycological examination of the wound was negative.

Figure 1. Integumentary squamous cell carcinoma in an eight-year-old, female cockatiel (*Nymphicus hollandicus*) before chemotherapy.

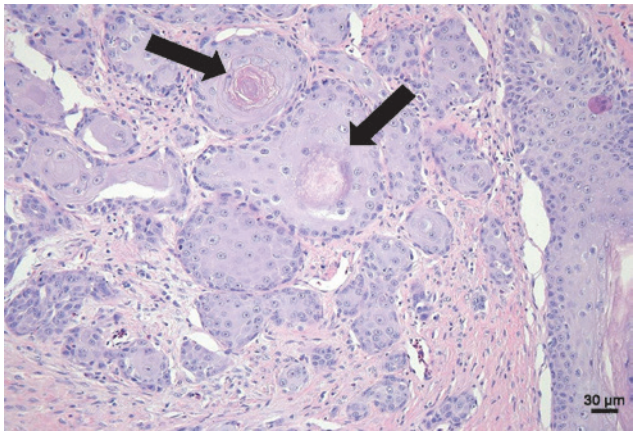

Figure 2. Histopathological examination of squamous cell carcinoma prior to carboplatin administration: strands of tumor cells surrounding keratin pearls (black arrows).

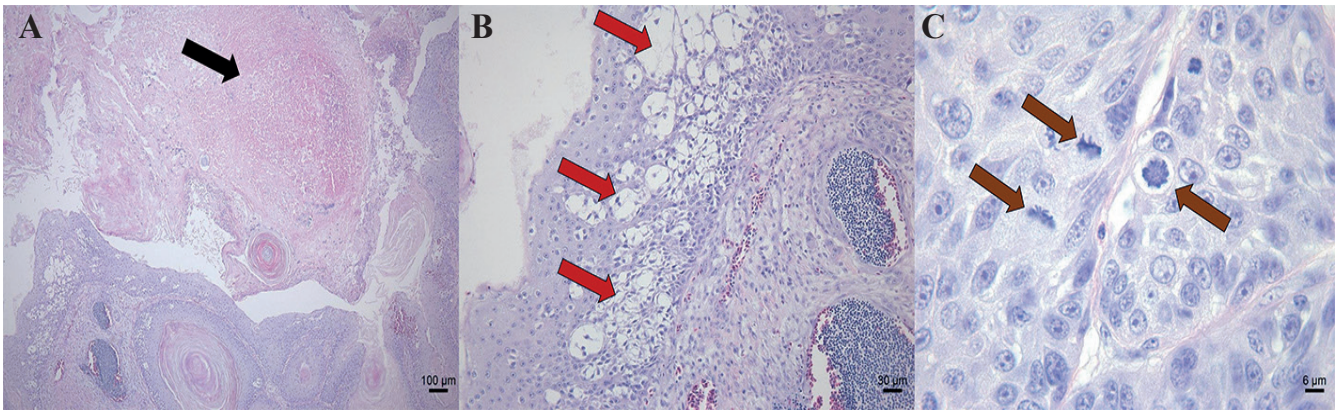

Figure 3. Histopathological examination of squamous cell carcinoma at necropsy (following carboplatin treatment) showed A. tumor necrosis (black arrows), B. vacuolar degeneration (red arrows) and C. mitotic catastrophe (brown arrows).

Four weeks postoperative, the clinical condition of the bird was improved, the bodyweight increased (95 g), and the bird started moulting, characterized by the appearance of the normal white to light yellow feathers. The tumor however, had reoccurred at the side of the non-healing surgical wound. Blood hematology and biochemistry showed no aberrant values apart from an increased creatine kinase level of 961 U/L (reference value: 30-245 U/L, Carpenter, 2012), which indicates leakage of the enzyme from muscle cells, often as a result of inflammation or trauma. Intratumoral administration of carboplatin (1.5 mg/cm³) was started on a weekly basis. Carboplatin was administered during general inhalation anesthesia with isoflurane as previously described, and analgesia was provided using buprenorphine (0.1 mg / kg BW IM). Carboplatin (Carboplatin Hospira Onco-Tain® injection 10 mg/ml, Hospira, Belgium) was mixed 1:1 with sterile filtered sesame oil (Sigma-Aldrich, Belgium). The length (L), width (W) and height (H) of the tumor were measured and the volume was calculated at 0.7 cm³ based on the formula "L x W x H x Pi / 6". Because the carboplatin solution would only diffuse 2-3 mm, a grid was created on the tumor with injection points being spaced every 5 mm, resulting in an overlap of the radius from adjacent points. For the administered dosage of 1.5 mg/cm³, this resulted in 0.02 ml being injected per infiltration site. One week after the initial carboplatin administration, the tumor was still growing and measured 1.56 cm³. A second intratumoral administration of carboplatin was done following the same protocol. Because of further tumor progression after the second administration of carboplatin, the decision was made to surgically resect the mass before the next infiltration chemotherapy. However, the bird died during general anesthesia prior to the start of this procedure.

A complete post-mortem examination of the cockatiel was performed. The SCC measured 1.86 cm³. The superficial part of the tumor was black and showed an ulcerative appearance. Furthermore, macroscopic examination at necropsy revealed mild atrophy of pectoral muscles, congestion of lungs and liver and hepatomegaly. Histopathological examination of the tumor showed a similar image as the initial mass. However, large zones of necrosis (Figure 3A) and bacterial colonization were seen in the tumor, and there was infiltration with a limited number of lymphocytes and plasma cells. At different locations in the tumor, there was vacuolar to hydropic degeneration of the neoplastic cells next to a distinct intercellular edema (Figure 3B). The mitotic index was higher than in the sections of the initially removed mass (8 mitotic figures per HPF), but these were mainly atypical mitotic figures. This form of cell death is termed mitotic catastrophe (Figure 3C). Immunohistochemistry of the tumor was performed both on sections of the surgically removed tumor before intratumoral carboplatin injection, and on sections of the tumor at necropsy. Clumps of cytokeratin positive cells were seen in the tumor before chemotherapy but also during the treatment. An E-cadherine immunohistochemistry test suggested the absence of apoptosis of the neoplastic cells, indicating vacuolar to hydropic degeneration of cells. Histology of the other tissues showed no metastatic disease but the lungs to be diffusely congested and containing several small aggregations of melanocytes. Multifocal epithelization of the Bowman capsule were observed in the kidney. The liver was diffusely congested. A multifocal, mild vacuolization of the cytoplasm of the hepatocytes was observed. Additionally, the hepatocytes contained a brown granular pigment and no signs of metastasis were found. Multifocal nests of heterophils and hemosiderin-loaded macrophages were present in the liver parenchyma. The white pulp of the spleen consisted mostly of lymphoblasts and macrophages. The red pulp was relatively anemic and contained many macrophages, some of them were hemosiderin-loaded.

To support further diagnostics of the underlying chronic liver failure, additional tests were performed. The PCR test for chlamydia of liver tissue, which was stored at -20°C after necropsy, was negative. To test hypovitaminosis A, the level of vitamin A was deter-

mined by high performance liquid chromatography (HPLC) in the liver tissue (Medilab, Ghent, Belgium), and was considered to be normal (405.9 µg/g tissue, Friedman et al., 1991).

DISCUSSION

This case report shows an integumentary SCC in a cockatiel treated with intratumoral administration of carboplatin following surgical excision. Surgical excision of a SCC is considered to be the first-choice therapy (Koski, 2002; Filippich, 2004; Reavill, 2004; Malka et al., 2005; Klaphake et al., 2006). Taking into account the aggressive local invasion of a SCC, a surgical margin of 1 cm is suggested in order to achieve a complete excision (Lightfoot, 2010). Similar as in this case, neoplastic processes in birds are often presented in an advanced stage; therefore, complete tumor excision is not always possible. Tumor localization on the wing made complete excision impossible in this case. Wing amputation could have been considered as an alternative treatment approach (Filippich, 2004). However, this was declined by the owner. Since birds are highly inclined to use flight as means of locomotion, wing amputation might negatively affect animal welfare (Harris, 1997). Another disadvantage of wing amputation might be the occurrence of keel trauma in birds attempting to fly and hitting the floor or other surfaces (Zhang et al., 2011). In a survey of 85 cases of SCC in birds, Zehnder et al. (2014) found an odds ratio of 7.48 for complete or partial response in patients that had complete surgical excision. Complete surgical excision of a SCC yields the best hope for cure.

Since complete tumor excision is not always possible, a combination of different treatment strategies should be taken into account to improve treatment outcome (Manucy et al., 1998; Filippich, 2004; Graham et al., 2004; Mehler and Bennett, 2004; Mauldin and Shiomitsu, 2005; Lightfoot, 2010). Chemotherapy may be used in addition to surgical excision, with or without radiation therapy or cryosurgery (Meleo, 1997; Filippich, 2004; Graham et al., 2004; Filippich and Charles, 2004; Ferrel et al., 2006). Cisplatin, carboplatin and 5-fluorouracil have been used empirically for the treatment of SCCs in birds (Filippich, 2004). Three cases of oral SCC and one case of a submandibular SCC in psittacine birds showed partial remission after intratumoral administration of cisplatin and a combination with cryotherapy. The species involved were an African grey parrot (*Psittacus erithacus*), an eclectus parrot (*Eclectus roratus)*, a green wing macaw (*Ara chloroptera*) and a Wagler's conure (*Aratinga wagleri*) (Lightfoot, 2010). Manucy et al. (1998) reported the unsuccessful treatment of concurrent usage of a SCC of the mandibular beak in a Buffon's macaw (*Ara ambigua*) with the combination of Cobalt-60 radiation therapy and intratumoral cisplatin administration. In the present case, carboplatin was selected instead of cisplatin, taking into account the lower toxicity of carboplatin compared to cisplatin, with a similar mode of action and efficacy as observed in human medicine (Ito et al., 2013; Dilruba and Kalayda, 2016). However, carboplatin is less potent than cisplatin; therefore, depending on the type of cancer, the dosage of carboplatin is usually four times higher than that of cisplatin to achieve similar effectiveness. This difference is related to the kinetics of the aquation reactions of cisplatin and carboplatin and their subsequent reactions with DNA (Knox et al., 1986). Taking into account the patient's suspected history of chronic liver disease and the risk of carboplatin-induced hepatotoxicity in the present case, intratumoral chemotherapy was preferred over intravenous or intra-osseous administration (Manucy et al., 1998; Filippich, 2004; Kent, 2004; Vail and Thamm, 2005). It has been suggested that intratumoral administration of chemotherapy has the benefit to reach a higher drug concentration at the tumor site with less systemic side effects (Manucy et al., 1998; Filippich, 2004; Graham et al., 2004; Filippich and Charles, 2004). Due to their radiosensitization effect, simultaneous administration of cisplatin or carboplatin and radiation therapy might improve therapeutic success (Meleo, 1997; Manucy et al., 1998; Filippich, 2004; Graham et al., 2004; Filippich and Charles, 2004; Vail and Thamm, 2005). However, radiation therapy was not added to the cockatiel's treatment protocol because of financial constraints.

Carboplatin dosage was selected based on a study in cats with a SCC at the *planum nasale,* which were successfully treated with intratumoral carboplatin in purified sesame oil at a dosage of 1.5 mg/cm³ with an interval of ten to fourteen days between different administrations (Theon et al., 1996; Vail and Thamm, 2005). Chemotherapy in birds has not been widely used, and therefore, treatment protocols are mostly extrapolated from other animal species (Filippich, 2004; Kent, 2004; Filippich and Charles, 2004; Hahn, 2005). Mixing carboplatin with a collagen matrix or a water in sesame oil emulsion, which is more cost effective, prevents a fast diffusion of the drug out of the neoplastic tissue (Graham et al., 2004). Following intravenous or intra-osseous administration, carboplatin has longer persisting levels of free plasma platinum than cisplatin, due to a higher mean $T_{1/2el}$ of free plasma platinum in sulphur-crested cockatoos (*Cacatua galerita*) (Filippich et al., 2000; Filippich et al., 2004). For carboplatin, the mean $T_{1/2el}$ of free plasma platinum counts 1.0 ± 1.7 hours and for cisplatin 0.413 ± 0.122 hours only. This difference in mean $T_{1/2el}$ is probably a result of a difference in body clearance between carboplatin (5.5 mL/min/kg BW) and cisplatin (11.7 mL/min/kg BW) (Filippich et al., 2000; Filippich et al., 2004). Recently, Antonissen et al. (2015) demonstrated by allometric scaling a clear correlation ($R^2 > 0.97$) between BW and the elimination half-life ($T_{1/2el}$) of carboplatin in different avian species, i.e. budgerigar, pigeons, ducks and chickens,

expressed by the formula: $T_{1/2el\ carboplatin} = 0.1147$ (log value of BW)$^{0.3046}$. The highest platinum accumulation after carboplatin administration occurs in the kidneys and liver, and is comparable with the concentrations found after administration of cisplatin (Filippich et al., 2004; Filippich and Charles, 2004; Filippich et al., 2005). However, intratumoral platinum accumulation and the carboplatin elimination rate following intratumoral administration are unknown. Similarly to this case, an amazon parrot with a integumental SCC was treated with surgical debulking and intratumoral carboplatin at a dosage of 5 mg/kg BW in a 10 mg/ml water in sesame oil emulsion, with a temporary growth interruption of the SCC (Wilson et al., 2000).

No clinical signs of carboplatin toxicity were observed in this case. However, blood hematology and biochemistry were not evaluated after the administration of carboplatin. Filippich et al. (2005) observed anorexia and vomiting in cockatoos following to carboplatin administration at a dose of 5 mg/kg BW. Myelosuppression is a dose limiting side effect of carboplatin. However, this has only been described in avian cases where carboplatin was administered at multiple doses of 11.25-15 mg/kg BW (Filippich, 2004; Childs-Sanford et al., 2006). Nevertheless, histopathology of the spleen revealed that the red pulp was relatively anemic, but with many macrophages. In comparison to cisplatin, carboplatin has a less pronounced neurotoxicity and ototoxicity when used in human patients (Kelland, 2007; Thomas and Chatelut, 2007; Dilruba and Kalayda, 2016).

Despite the increase in tumor size, histopathological evaluation showed vacuolar to hydropic degeneration. The presence of mitotic catastrophe (atypical mitotic figures) and necrosis of the tumor are both suggestive of the effectiveness at the cellular level of the chemotherapy. Mitotic catastrophe is a cellular process of eliminating eukaryote cells, which are incompetent in proceeding a normal mitosis. This process finally leads to apoptosis, necrosis or senescence in case of cells not being able to repair the DNA or mitochondrial damage. If mitotic catastrophe is enhanced using therapeutic products, it may finally lead to the interruption of tumor growth. DNA-damage induced by platinum analogues is known to result in mitotic catastrophe. Multinucleation is another histopathological sign of mitotic catastrophe (Vitale et al., 2011). The noticed vacuolar to hydropic degeneration in the present case are findings seen with oncosis, which lead to necrosis and cell death (Majno and Joris, 1995).

CONCLUSION

In this report, a case of SCC is presented where intratumoral administration of carboplatin following surgical excision was used as treatment protocol, after tumor regrowth was seen with the previous treatment. Despite the increase in the tumor size, histopathological evaluation showed vacuolar to hydropic degeneration. However, further knowledge on the efficacy and safety of carboplatin after systemic and intratumoral administration is lacking in birds. This case report demonstrates that dose extrapolation of chemotherapeutic agents from other animal species does not guarantee success. The observed vacuolar to hydropic degeneration and the presence of mitotic catastrophe following carboplatin treatment are suggestive for the effectiveness at cellular level of the chemotherapy. Therefore, a higher dosage, another administration route or combination with radiation therapy should be considered. Regular blood hematology and biochemistry are recommended in order to follow-up possible side-effects of chemotherapy.

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Peripheral osteoma of the mandible in a cat

Perifeer osteoma van de mandibula bij een kat

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ABSTRACT

A six-year-old, male, castrated domestic shorthair cat was presented for evaluation of an oral mass. Full physical examination revealed a large hard mass arising from the lateral aspect of the caudal part of the left mandible. Abdominal ultrasound and thoracic computed tomography (CT) were performed to rule out metastatic disease. In addition, CT of the skull with intravenous contrast agent was performed. Histopathological examination of extra-oral biopsies confirmed the tentative diagnosis of peripheral osteoma. A three-dimensional printed skull was used in conjunction with the CT images to determine the detailed location and extent of the mass and to identify anatomical spatial relationships with important neurovascular structures. Surgical debulking of the osteoma was performed. The cat recovered uneventfully but the mass regrew nine months after debulking therapy, sooner than expected. Nevertheless, CT scan of the skull was suggestive for regrowth of the peripheral osteoma rather than for malignant transformation. The owners did not elect to pursue caudal mandibulectomy.

SAMENVATTING

Een zesjarige, mannelijke, gecastreerde kat werd aangeboden voor evaluatie van een orale massa. Op het lichamelijk onderzoek werd een grote harde massa opgemerkt aan de laterale kant van het caudale deel van de linkermandibula. Abdominale echografie en computertomografie (CT) van de thorax werden uitgevoerd om metastasen uit te sluiten. Bijkomend werd een CT-scan van de schedel met intraveneuze contrastvloeistof genomen. Histopathologisch onderzoek van extraorale biopsieën bevestigde de waarschijnlijkheidsdiagnose van perifeer osteoma. Een driedimensionale (3-D), geprinte schedel in combinatie met CT-beelden werd gebruikt om de exacte locatie en omvang van de massa te beoordelen en om alle belangrijke neurovasculaire structuren te kunnen onderscheiden. Chirurgische debulking van het osteoma werd uitgevoerd. De kat herstelde zonder problemen, maar de massa kwam negen maanden na debulking terug. Dat was eerder dan verwacht. Desondanks was de CT-scan van de schedel suggestiever voor terugkeer van het perifere osteoma dan voor een maligne transformatie. De eigenaren kozen ervoor om niet verder te gaan met caudale mandibulectomie.

INTRODUCTION

In domestic animals, primary bone tumors are infrequently diagnosed. In cats, the incidence is estimated to be 4.9 per 100,000 individuals (Dernell et al., 2007). However, most primary bone tumors in cats are malignant (67-90%) (Dernell et al., 2007; Haynes et al., 2012; Thompson and Dittmer, 2016). The mean age at which bone tumors develop in cats is eight to ten years (Dernell et al., 2007; Haynes et al., 2012). Long bones are more often affected than the axial skeleton (Dernell et al., 2007), with only 6% of all primary bone tumors arising from bones of the skull (O'Brien et al., 1996).

Figure 1. Three-dimensional computed tomographic reconstruction of the head of a six-year-old, male, castrated domestic shorthair cat. The images show a solitary osseous mass, firmly attached to the caudal aspect of the left mandible.

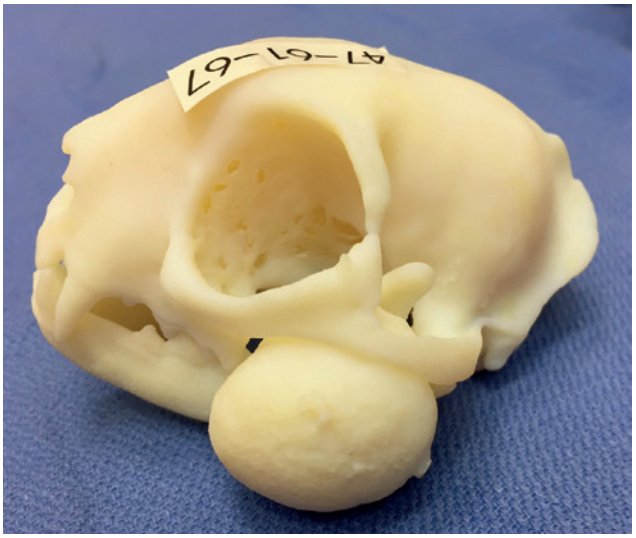

Figure 2. Three-dimensional reconstruction of the skull. The egg-sized mass is firmly adhered to the underlying bone of the left mandible and extends laterally along the mandibular bone. The caudodorsal extension ends immediately rostral to the temporomandibular joint.

Osteoma is a benign primary bone and cartilage tumor (Pool, 1978; Foley, 1993) that is characterized by continuous, slow growth (Mittal and Iyer, 2008) of cancellous bone (trabeculae), compact bone (dense lamellae) or a combination of both (Woldenberg et al., 2005). Osteomas can occur in all domestic animals, but they are more frequently diagnosed in horses and cows than in other species (Pool, 1978). In an early review article it has been reported that less than 2% of cats diagnosed with bone tumors are affected by osteoma (Quigley and Leedale, 1983). Osteomas usually arise from flat bones, with a predilection for the mandible, maxilla, nasal sinuses and the craniofacial region (Foley, 1993; Linden et al., 2016). In cats, the caudal portion of the mandible is the most frequently affected region (Fiani et al., 2011).

In humans, osteomas are classified based on their origin as peripheral, central and extra-skeletal (Johann et al., 2005). Peripheral osteomas grow on the surface of bones, whereas central osteomas arise from within the bone, and extraskeletal osteomas originate in muscle tissue (Woldenberg et al., 2005). Although a similar classification does not yet exist in veterinary medicine, two distinctive patterns resembling peripheral and central osteomas have been distinguished on computed tomography (CT) scans (Fiani et al., 2011).

In contrast to malignant primary bone tumors, the prognosis of osteoma after complete surgical excision is generally good. The prognosis of central osteoma is more reserved than for the peripheral variety because it is known to recur more frequently than peripheral osteoma and excisional surgery is more invasive (Larrea-Oyarbide et al., 2008). When the tumor is in an advanced stage, the extent of the surgery may be so radical that the patient's quality of life would be severely compromised. Therefore, it is important to diagnose and treat an osteoma in its early stage (Corgozinho et al., 2015).

In the current case report, an adult cat with a mandibular peripheral osteoma treated by surgical debulking is described.

CASE REPORT

A six-year-old, male, castrated, domestic shorthair cat (9.2 kg) was presented to the William R. Pritchard Veterinary Medical Teaching Hospital (VMTH) Dentistry and Oral Surgery Service (DOSS), University of California, Davis for evaluation of an oral mass. The owner presented the cat to his private practitioner approximately two months earlier, for evaluation of a visible swelling of the face that had been present for an unknown period of time. Physical examination revealed a round, egg-sized mass lateral to the caudal half of the left mandible. The veterinarian attempted to obtain a fine-needle aspirate, but was unsuccessful due to the hard texture of the mass. On radiographs, the bony nature of the egg-shaped mass was demonstrated. The cat was subsequently referred to a local hospital for CT scan of the skull and biopsies of the mass. A tentative diagnosis of osteoma was made since histopathology only revealed normal woven bone. The cat was subsequently referred to the UC Davis DOSS VMTH.

The owner reported that despite having a large mass, the cat was able to eat, drink and groom itself normally. Physical examination, blood work, urine collection and abdominal ultrasound were performed and were unremarkable. On oral examination, a firm mass was noted at the left caudal oral cavity, causing the side of the face to bulge. The mucosa overlying the mass was mottled red-pink and mildly ulcerated in patches. Moderate gingivitis with mild diffuse plaque and calculus accumulation was noted.

The next day, the cat was placed under general anesthesia. Thoracic CT demonstrated no evidence of metastatic disease or other comorbidities. CT of the skull with intravenous contrast agent revealed a 2 by 3

by 4 cm, smoothly margined, homogenously mineral dense mass arising from the left mandible caudal to the fist molar tooth (Figure 1). The mass had a pedunculated extension laterally along the mandible from the level of the left mandibular fourth premolar tooth to immediately rostral to the temporomandibular joint. Surrounding the mass, there was a halo of soft tissue density with peripheral contrast enhancement. The mass displaced the left masseter muscle medially and bulged ventral to the zygomatic arch to impinge on the ventral aspect of the left pterygopalatine fossa. The left eye was mildly displaced dorsolaterally. A three-dimensional model of the skull was printed for surgical planning (Figure 2). Based on the oral examination and CT findings, an osteoma of the left mandible was suspected. Other differentials included osteosarcoma and osteochondroma.

Dental radiographs confirmed the large bone-opaque mass arising from the left mandible. An incisional biopsy was obtained. Over the ventrolateral aspect of the mass, down to the level of bony tissue, an approximately 2.5-cm skin incision was made. The subdermal tissue and masseter muscle were bluntly dissected to enhance the visualization of the mass. Using an osteotome and mallet, a bone biopsy sample was collected. The surgical site was irrigated with sterile saline 0.9%. The masseter muscle was apposed with simple-interrupted sutures using poliglecaprone 25 4-0 (Monocryl®, Ethicon, US). The same suture was used for the intradermal layer, with the knot buried. Surgical skin glue was used for final skin apposition, as skin sutures would require heavy sedation to remove given the patient's temperament. The biopsy sample was placed in 10% buffered formalin and submitted for histopathology. Photographs were obtained postoperatively to demonstrate the location of the skin incision, which would need to be included in more definitive treatment. Awaiting the biopsy results, buprenorphine (Buprenex®, Reckitt & Colman, UK) 0.01 mg/kg was administered sublingually three times a day for the next three days. Amoxicillin and clavulanic acid (Clavamox®, Zoetis, US) 12.5 mg/kg per oral (PO) two times daily was administered while the incision was healing. It was also recommended to feed soft food, continue keeping the cat indoors, and for the patient to wear an Elizabethan collar to prevent self-trauma to the healing biopsy site.

Histopathological findings revealed bone that was composed of a dense network of lamellar and woven bone, forming expansive and disorganized trabeculae and islands. The surfaces of the trabeculae were variably lined by a single row of osteoblasts that rarely piled up two to three rows thick. The intertrabecular connective tissue was relatively paucicellular. Multifocally, there were vascular canals. Mitotic figures were not noted. The tentative diagnosis of osteoma was confirmed and surgical debulking of the osteoma was advised.

On the day of surgery, the cat was anesthetized and placed in dorsal recumbency under anesthesia.

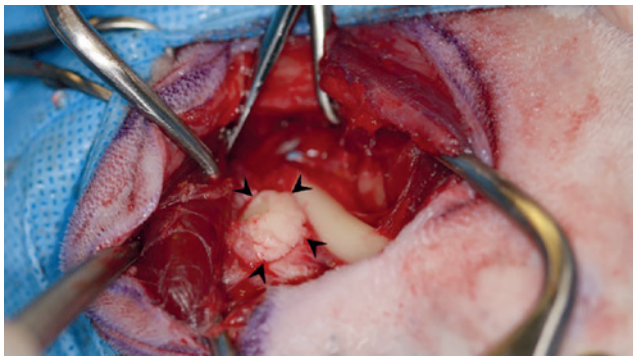

Figure 3. The overlying masseter and digastric muscles were dissected away, along with fibrous tissue, in order to improve visualization of the mass (arrow heads).

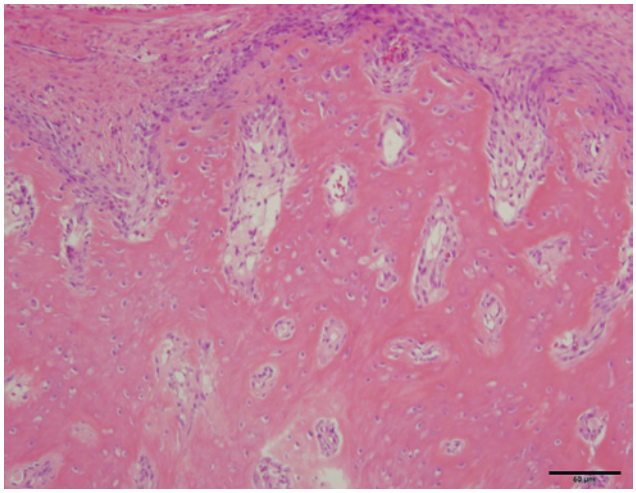

Figure 4. Photomicrograph of the mandibular osteoma of the cat. Note the mass is composed by fronts of woven bone lined by osteoblasts intimately interacting with connective tissue components lining the periphery of the mass (top of image). Also noted progressive maturation of woven bone towards the deeper portions of the mass (bottom of image). H&E stain; X10 magnification.

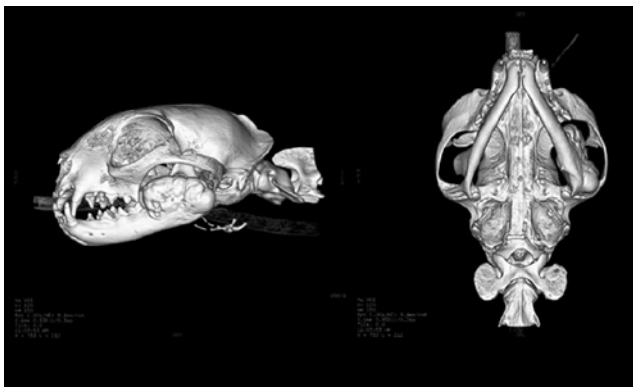

Figure 5. Three-dimensional computed tomographic reconstruction of the head of the domestic shorthair cat nine months after surgical debulking of an osteoma. The tumor recurred at the left caudal mandible with a more axial extension. The medial cortex immediately adjacent to the mass is severely thinned.

A fusiform skin incision was made overlying the left mandible. The masseter and digastric muscles and the fibrous tissue overlying the osteoma were bluntly and sharply dissected away from the surface of the mass (Figure 3). Using an osteotome and mallet, the mass was separated from the underlying mandible. A vessel "feeding" the mass was ligated twice with polyglactin 910 3-0 (Vicryl®, Ethicon, US) and transected. The osteoma was removed in a single piece, placed in 10% buffered formalin and submitted for histopathology. The underlying mandibular bone was further exposed and irregular bone was smoothed with a piezosurgery unit (Implant Center 2, Acteon, France). The surgical site was irrigated with sterile saline 0.9%. The deep and superficial muscle layers were apposed using poliglecaprone 25 4-0 in a simple-interrupted pattern. The overlying skin was closed in the same pattern with nylon 4-0 (Ethilon®, Ethicon, US), tacking most sutures into the underlying superficial muscle and fascia to reduce dead space and further reinforce the closure.

The cat was hospitalized overnight and additional pain management was achieved with meloxicam (Metacam®, Merial, Germany) 0.2 mg/kg IV at extubation and oxymorphone 0.05 mg/kg IV every six hours.

Histopathology revealed that the submitted tissue consisted of 80% immature woven bone (Figure 4). The margins were variably lined by pallisading osteoblasts. Superficially, the woven bone was interwoven with an overlying thin fibrovascular capsule. The clinical diagnosis of a periosteal osteoma was confirmed.

Nine months after debulking surgery, the owner returned to the UC Davis DOSS with the complaint that the mass had regrown. In the owner's opinion, the progression was slow. On physical examination, a bony mass attached to the left caudal mandible was appreciated, yet smaller than the previously diagnosed osteoma and potentially wrapping around slightly to the medial aspect. A cone beam CT scan was performed and showed a mass with a more axial extension than the osteoma of the year before and no impinging on the pterygopalatine fossa (Figure 5). The left caudal mandible was more severely affected by the mineralized mass than the year before. The mass expanded the affected portion of the mandible, causing severe thinning to complete loss of the medial cortex along the length of the mass.

Caudal mandibulectomy was discussed as the curative treatment of choice given the marked thinning of the cortical bone and hence the risk of mandibular fracture if debulking was considered for a second time. However, the cat was free of clinical signs and the owner elected not to proceed with additional surgery. The owner was advised that humane euthanasia should be considered if the cat's quality of life deteriorated. The owner was warned to watch for signs of diminishing quality of life, including decreased appetite or ability to prehend or masticate food, and temporomandibular joint ankylosis.

DISCUSSION

In this case report, a six-year-old domestic shorthair cat with a peripheral osteoma of the mandible is described. Based on previously published single case reports and one case series in cats, there does not seem to be a breed predilection; however, it appears that neutered males are affected more often than females (Maas and Theyse, 2007; Fiani et al., 2011; Corgozinho et al., 2015). The age of cats at presentation is widespread (1-23 years), although most diagnoses of peripheral osteoma in cats are reported around the age of nine years.

The clinical presentation of maxillofacial osteoma can be highly variable, ranging from asymptomatic to difficulties with eating, mandibular drift, mandibular swelling, fever, epiphora, and exophthalmos (Fiani et al., 2011). The cat in the current case report did not have any problems associated with the mass at the time of either presentation, but the owner consulted his primary care veterinarian due to an obvious facial asymmetry.

In both domestic animals and humans, osteomas are mostly described in the maxillofacial region (Vigorita et al., 2008; Thompson and Dittmer, 2016). They are characterized by continuous growth of bone, but can become quiescent for years. Peripheral osteomas can remain unnoticed for an unknown period of time, unless they begin to interfere with important adjacent anatomical structures due to their size (Thompson and Dittmer, 2016).

The mandible was the bone of origin in five out of seven cats in a case series of osteomas (Fiani et al., 2011). In single case reports, osteomas in cats in the nasal cavity, the maxilla, the zygomatic arch, the temporomandibular joint, periorbital, the ribs and the radius have been described (Pool, 1978; Foley, 1993; Maas and Theyse, 2007; Fiani et al., 2011; Haynes et al., 2012; Corgozinho et al., 2015; Cunha et al., 2015).

There is ongoing debate whether osteoma is a true neoplasm, a developmental anomaly or an osteogenic lesion triggered by trauma or infection (Sayan et al., 2002; Woldenberg et al., 2005; Boffano et al., 2012). In the case described here, a traumatic cause exacerbated by muscle traction could have been the trigger for osteoma formation, since the peripheral osteoma was located along the buccal aspect of the left mandible, a region susceptible to trauma, as described in the human literature (Sayan et al., 2002). However, the owner did not recall any history of trauma.

The diagnosis of peripheral osteoma should be made based on the combination of clinical findings, diagnostic imaging and histopathology (Fiani et al., 2011). In the case reported here, the only significant clinical abnormality was a marked bulging of the left side of the face. Usually, peripheral osteomas are not painful on palpation (Cunha et al., 2015). Diagnostic imaging of peripheral osteoma is based on radiological examinations or CT scan (Mittal and Iyer, 2008).

Conventional radiography typically shows a pedunculated, mushroom-shaped, radiopaque mass (Durão et al., 2012). Although the radiological features are suggestive, CT is preferred to understand the precise location and extent of the mass, as well as acting as an aid for surgical planning (Mittal and Iyer, 2008; Durão et al., 2012). On CT, a peripheral osteoma is usually characterized by bone density, smooth margins and a sessile base (compact peripheral osteoma) although it can be less hyperattenuating with rather irregular margins and attached to the bone via a pedicle (cancellous peripheral osteoma) (Richardson et al., 1999; Fiani et al., 2011; Thompson and Dittmer, 2016). Importantly, the definitive diagnosis of osteoma can only be made by proper histological examination (Durão et al., 2012; Thompson et al., 2016). Peripheral osteomas are histologically characterized by a deeper layer of lamellar bone trabeculae and a superficial layer of woven bone trabeculae (Linden et al., 2016; Thompson and Dittmer, 2016). There are two distinctive histological patterns of peripheral and central osteomas, the compact and the cancellous osteoma. Normal-appearing bone with limited marrow spaces and occasional Haversian canals is typical for compact osteoma. Cancellous osteoma consists of bone marrow with osteoblasts and trabeculae of bone (Richardson et al., 1999; Sayan et al., 2002). Based on CT findings and histopathology, the osteoma described in this case was a compact peripheral osteoma.

Two important lesions to be differentiated from osteoma and one another are ossifying fibroma and fibrous dysplasia. All three bony lesions mostly affect the craniomaxillofacial region (Linden et al., 2016). Other differential diagnoses for bone tumors reported in the literature are trauma-induced exostoses of the jaw, osteochondroma, and periosteal osteochondrosarcoma (Fiani et al., 2011; Haynes et al., 2012). Parosteal osteosarcoma (OSA) is another differential that should be taken into account in this specific case, since the mass in the cat described recurred sooner than expected in case of osteoma, which could indicate malignant transformation (Johann et al., 2005; Linden et al., 2016). It is important to note that malignant transformation of an osteoma has never been reported previously in any species (Pool, 1978; Sayan et al., 2002).

Mandibulectomy or debulking are two surgical treatment options that can be performed in cats with mandibular osteomas (Fiani et al., 2011). In humans, peripheral osteomas located on the mandible can be removed via an intraoral or an extraoral approach (Woldenberg et al., 2005; Starch-Jensen, 2017); the former being preferred for esthetic reasons (Woldenberg et al., 2005). The main disadvantage of an intraoral approach is decreased visualization and thus the higher risk of damaging adjacent anatomical structures. In the cat described, an extraoral approach was performed, as in all previously reported cases (Foley, 1993; Maas and Theyse, 2007; Fiani et al., 2011). Due to the complex anatomy of the maxillofacial region, preoperative planning is crucial (Winer et al., 2017). In this case, the authors opted for CT images that were used to create a three-dimensional (3-D) printed skull. The 3-D printing technique has been recently introduced into veterinary medicine to guide surgeons in their preoperative planning (Arzi et al., 2015; Harrysson et al., 2015). This technique makes it possible to convert a two-dimensional (2-D) image (e.g. CT) into a 3-D object (e.g. skull model) in order to achieve a better understanding of spatial relationships and patient-specific anatomy (Winer et al., 2017). This technique reduces the risk of complications (e.g. nerve damage, hemorrhage) (Winer et al., 2017) and may reduce surgery time (D'Urso et al., 1999). Three-dimensional printing is a useful tool for preoperative planning, but it still differs from intraoperative anatomy since it does not include soft tissues (Winer et al., 2017). Due to the location of the mass presented in this study, involving the ramus and body of the mandible and the close proximity to the temporomandibular joint, tumor-free margins could not be obtained without performing a unilateral caudal mandibulectomy.

The osteoma regrew nine months after the debulking surgery, which was not entirely unexpected since microscopic remnants were known to be left behind at the time of debulking surgery. However, the interval from surgery to regrowth was shorter than expected. The long-term prognosis for the cat in this case report is unclear since osteomas near the temporomandibular joint can induce temporomandibular joint ankylosis (Maas and Theyse, 2007). The surgical option of caudal mandibulectomy to achieve definitive cure was explained to the owner, but he elected to forgo further surgery.

CONCLUSION

Clinicians should not jump to conclusions when confronted with bony proliferations; a conclusive rather than a tentative diagnosis should be made before discussing the treatment options and long-term prognosis with the owner. Although relatively rare in dogs and cats, osteoma should be included in the list of differential diagnoses of bone tumors. Osteomas are benign tumors with a typically slow growth rate and generally good prognosis. They may be treated by either debulking surgery or curative radical excision, depending on the location, goals of the client and effect on the patient's quality of life.

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Incomplete ossificatie van de humeruscondylen (IOHC) bij de hond

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SAMENVATTING

Incomplete ossificatie van de humeruscondylen (IOHC) bij de hond is een vrij zeldzaam elleboogprobleem dat voornamelijk bij spaniëlrassen wordt gezien maar ook bij andere rassen kan voorkomen. Deze aandoening gaat niet altijd gepaard met klinische symptomen maar kan de aanleiding zijn van elleboogkreupelheid en zelfs condylaire humerusfracturen. De diagnose van IOHC is niet altijd eenvoudig. Radiografisch is de fissuur niet altijd zichtbaar. Computertomografie is de meest efficiënte beeldvormingstechniek om deze aandoening met zekerheid te diagnosticeren. Indien het dier geen klachten vertoont, kan conservatieve therapie worden overwogen maar in geval van kreupelheid is het plaatsen van een compressieschroef de meest gangbare behandeling.

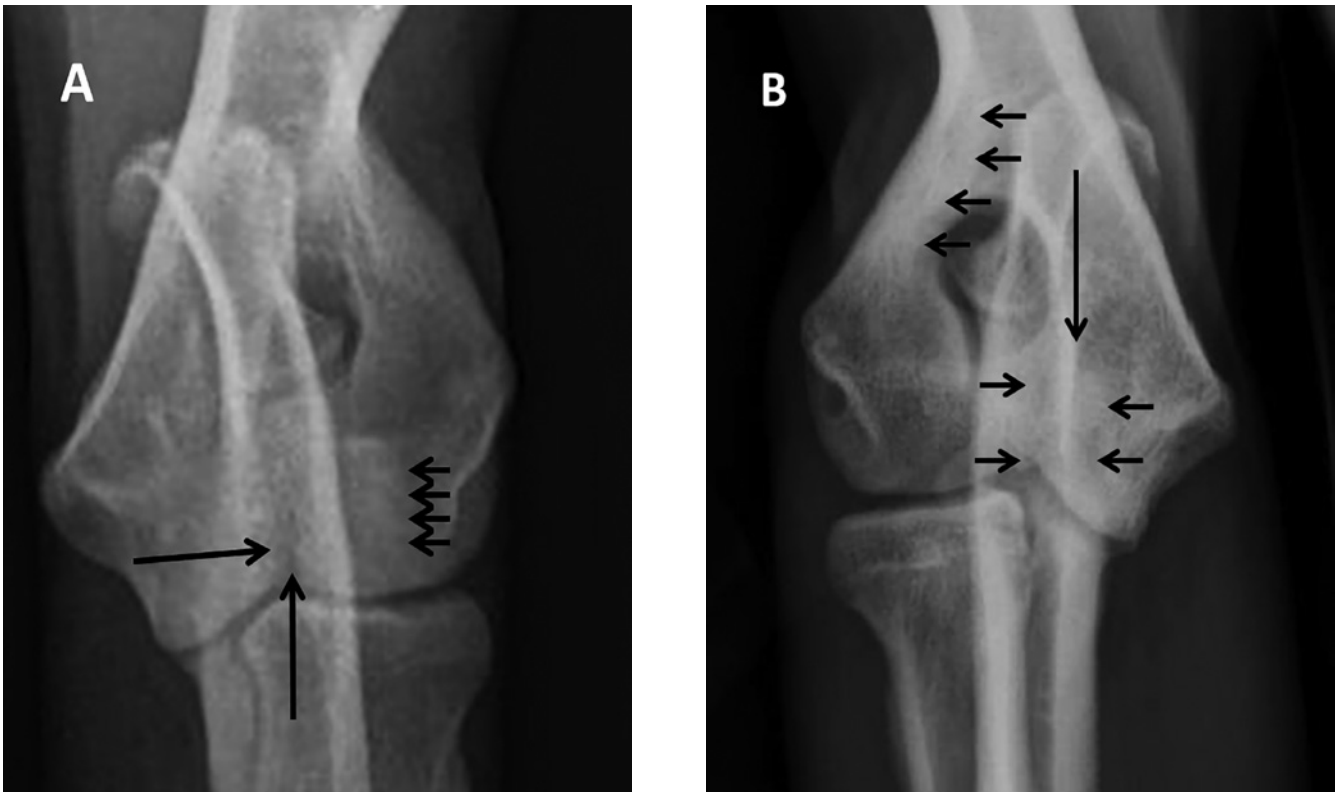
INLEIDING

In de praktijk komen elleboogproblemen vaak voor, meestal bij opgroeiende honden van grote en reuzenrassen (Cook, 2001; Scott en Witte, 2011). Er zijn verscheidene aandoeningen die kreupelheid in de elleboog kunnen veroorzaken, waaronder een reeks traumatische letsels, zoals fracturen en luxaties (McKee et al., 2005; Sajik et al., 2016), degeneratieve letsels, zoals flexor enthesiopathie (De Bakker et al., 2013), "medial compartment syndrome" (Coppieters et al., 2015), artrose en een reeks congenitale en ontwikkelingsstoornissen, zoals elleboogdysplasie. Elleboogdysplasie is de verzamelnaam voor vier aandoeningen: osteochondrose van de mediale humeruscondyl, losse processus anconeus (LPA), elleboogincongruentie en losse processus coronoïdeus (LPC). In de recente literatuur worden afwijkingen van de processus coronoïdeus "medial coronoid disease" (MCD) genoemd en als de voornaamste oorzaak van elleboogkreupelheid aanzien (Samoy et al., 2006; Villamonte-Chevalier et al., 2015).

Een meer zeldzame elleboogaandoening is incomplete ossificatie van de humeruscondylen (IOHC), waarbij er door het niet-fusioneren van de laterale en mediale humeruscondyl een fissuur of barst tussen de twee condylen blijft bestaan. De distale humerus ontstaat uit een fusie van drie ossificatiecentra: een groter mediaal en een lateraal centrum en een kleiner centraal centrum dat deel uitmaakt van de mediale humeruscondyl. De fissuurlijn is gelokaliseerd ter hoogte van de kraakbeengrens tussen de laterale en de mediale humeruscondyl, waarvan de fusie normaal op een leeftijd van acht à twaalf weken plaatsgrijpt (Moores, 2006). De kraakbeenplaat die tussen beide ossificatiecentra aanwezig is, loopt maar tot aan de groeiplaat van de distale humerus, terwijl de fissuur die men kan aantonen bij IOHC soms tot aan het foramen supratrochleare loopt. Dit foramen bevindt zich proximaal van de distale groeiplaat. Vermoedelijk loopt de fissuur verder door stressfracturen die ontstaan door reeds bestaande zwakheid van de humerus (Butterworth en Innes, 2001; Moores, 2006). Er is ook beschreven dat IOHC ontstaat ten gevolge van stressfracturen. Rondom de fissuurlijn is er sclerotisch beenweefsel aanwezig. Dit komt typisch voor bij stressfracturen en ondersteunt deze hypothese (Moores et al., 2012).

SIGNALEMENT

IOHC wordt vaker aangetroffen bij de spaniëlrassen, zoals de Engelse springer-spaniël en de cocker-spaniël, waarbij de aanleg erfelijk zou zijn (Marcellin-Little et al., 1994; Moores, et al., 2012). Deze aandoening komt ook voor bij de labrador-retriever, de rottweiler en ook bij andere, minder typische rassen (Moores, 2006; Favril et al., 2014). IOHC komt vaak bilateraal voor en meer bij reuen dan bij teven (Denny, 1983; Marcellin-Little et al., 1994; Moores, 2006). De dieren zijn meestal van middelbare leeftijd (Denny, 1983; Marcellin-Little et al., 1994; Butterworth and Innes, 2001) met een normaal lichaamsgewicht.



Figuur 1. Voor-achterwaartse opname van ellebogen: A. Doorheen de ulna is een fissuur zichtbaar die ongeveer halverwege tot aan het foramen supratrochleare reikt (grote pijlen). Rond de fissuur is er sclerose aanwezig (kleine pijltjes). B. Doorheen de ulna is vaag een fissuur waar te nemen bij deze vizsla (grote pijl). Rond de fissuur en ter hoogte van de laterale humerus zuil is er sclerose aanwezig (kleine pijltjes).

Figuur 2. Voor-achterwaartse opname van een elleboog waar een duidelijke mach-lijn te zien is (witte pijl). Deze mag niet verward worden met een fissuur die bij IOHC kan opgemerkt worden.

SYMPTOMEN

Incomplete ossificatie van de humeruscondyl kan ernstige pijn en kreupelheid veroorzaken. Als gevolg hiervan kunnen problemen ontstaan bij het trainen van werkhonden (Moores, 2006; Fitzpatrick, et al., 2009). Vaak breidt deze fissuur zich uit tot aan het foramen supratrochleare net proximaal van de groeiplaat, waarschijnlijk ten gevolge van een stressfractuur (Moores, 2006). Deze fissuur blijft een zwakke plek in de distale humerus en kan leiden tot een spontane fractuur, zelfs na een normale activiteit of na een gering trauma, zoals ergens afspringen. Het resultaat kan een eenvoudige fractuur zijn zoals een fractuur van de laterale condyl of een meer ingewikkelde zoals een bicondylaire fractuur (Fitzpatrick et al., 2009).

IOHC kan soms als een toevalsbevinding worden aangetroffen. In dat geval vertonen de honden geen symptomen.

DIAGNOSE

Om zeker te zijn van de diagnose van IOHC moet de intercondylaire fissuur zichtbaar zijn. Dit is op röntgenfoto's niet altijd het geval vanwege de inherente superpositie van de botstructuren. Op voor-achterwaartse radiografieën van uitstekende kwaliteit kan soms de fissuur zichtbaar zijn maar dan moet de röntgenbundel exact parallel lopen met de fissuurlijn. Daarom zijn meestal meerdere voor-achterwaartse

opnamen onder verschillende hoeken noodzakelijk. Deze worden niet routinematig uitgevoerd omdat de symptomen in veel gevallen niet altijd duidelijk zijn en deze aandoening niet overwogen wordt. De fissuurlijn kan partieel of compleet zijn, helemaal tot aan het foramen supratrochleare (Figuur 1). Het is belangrijk om een fissuurlijn ten gevolge van IOHC niet te verwarren met een mach-lijn (Figuur 2), een radiolucente lijn ter hoogte van de distale condylen die gecreëerd wordt door superpositie van twee botstructuren en die eigenlijk gezichtsbedrog is. Soms is er nieuwbeenvorming of een periostale reactie zichtbaar ter hoogte van de laterale zijde van de epicondyl en/of sclerose rond het foramen supratrochleare (Hoskinson en Tucker 2001; Cook en Cook, 2009) (Figuur 3).

Als er een vermoeden is van IOHC en indien het klinisch en radiografisch onderzoek geen uitsluitsel geeft, kan scintigrafie nuttig zijn om de lokalisatie in het ellebooggewricht te bevestigen. De contralaterale kant, die dikwijls ook aangetast is, kan tegelijkertijd onderzocht worden (Debruyn et al., 2013) (Figuur 4).

Computertomografie (CT) is de ideale techniek om een fissuur aan te tonen omdat superpositie van overliggende structuren vermeden wordt (De Rycke et al., 2002; Carrera et al., 2008). De fissuurlijn doorheen de distale humerus is duidelijk zichtbaar op de transversale beelden (Moores, 2006). Ze manifesteert zich als een translucente lijn met onregelmatige, sclerotische randen (Figuur 5 en 6). Er wordt aangeraden om de contralaterale kant ook te controleren vermits deze aandoening heel dikwijls bilateraal aanwezig is. Dikwijls worden ook elleboogincongruentie, afwijkingen van het coronoïd en artrose samen met IOHC in hetzelfde gewricht waargenomen (Carrera et al., 2008). Op CT-studies kunnen tevens andere elleboogproblemen aangetoond worden, zoals MCD, LPA en elleboogincongruentie. Ook artrose is duidelijk zichtbaar op CT en komt vaak voor bij ellebogen met IOH. De meest voorkomende lokalisatie van nieuwbeenvorming is de laterale zijde van de epicondyl. Bij uitgebreide artrose is nieuwbeenvoming aanwezig op de laterale en de mediale epicondyl (Carrera et al., 2008).

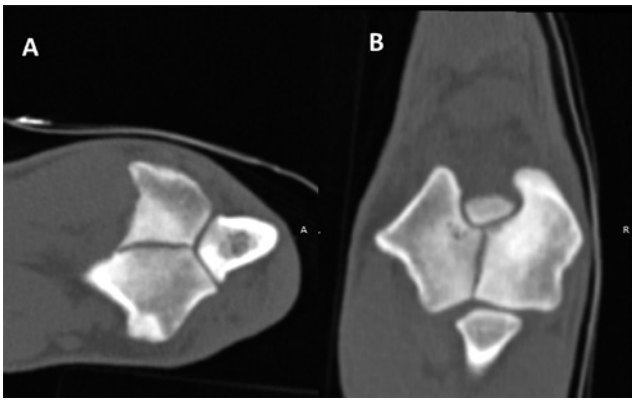
Bij aangetaste dieren kan elleboogartroscopie ook helpen bij de diagnose. In de meeste gevallen wordt een barst of fissuur in het gewrichtskraakbeen waargenomen op de overgang tussen de laterale en mediale humeruscondyl (Meyer-Lindenberg et al., 2002) (Figuur 7).

BEHANDELING

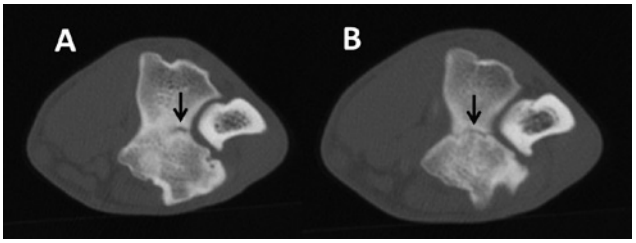
De behandeling van IOHC blijft controversieel. Conservatieve behandeling geeft meestal geen goed resultaat en resulteert op lange termijn meestal in een complete condylaire fractuur. Toch wordt meestal geen chirurgische behandeling aangeraden als er geen symptomen zijn en de diagnose enkel als toevalsbevinding wordt gesteld (Favril et al., 2014).

Figuur 3. A. Medio-laterale en B. voor-achterwaartse radiografieën van de rechterelleboog van een mannelijke, Engelse springerspaniël van zes maanden oud met IOHC en wisselende kreupelheid. A. Op de laterale opname ter hoogte van de distale humerus proximaal van het foramen intertrochleare is uitgesproken sclerose aanwezig (pijltjes). B. Op de voor-achterwaartse opname is de fissuurlijn amper te zien maar valt de erge sclerose rond het foramen intertrochleare op (pijltjes).

Figuur 4. Scintigrafisch onderzoek uitgevoerd bij een hond met bilaterale IOHC. In beide ellebogen is er een verhoogde opname van de radioactieve tracer (rode pijlen).



Figuur 5. A. Transversale en B. dorsale reconstructie. CT-beelden in botvenster van een rechterelleboog van een hond met IOHC. De fissuurlijn doorheen de distale humerus is duidelijk zichtbaar op de beelden. Ze manifesteert zich als een translucente lijn omgeven met onregelmatige, sclerotische randen.



Figuur 6. A. Transversale CT-beelden in botvenster ter hoogte van de distale humerus en B. meer distaal van een acht maanden oude vizla (cf. Figuur 1B). De fissuur (zwarte pijlen) kan een zeer discreet voorkomen hebben. Op het linkerbeeld is er sclerose rondom de fissuur.

In gevallen van pijn en kreupelheid is chirurgie wel aangewezen om de symptomen op te lossen en om preventief een complete fractuur te voorkomen.

De meest gekozen chirurgische behandeling is het plaatsen van een compressieschroef doorheen de twee condylen om het letsel te stabiliseren en de regio te versterken (Figuur 8). Een andere behandelingsmethode is het plaatsen van botenten in het defect. Er is echter nooit bewezen dat de resultaten hiervan beter zouden zijn dan bij de traditionele compressieschroef. Vaak blijft na behandeling van IOHC of van IOHC-geassocieerde fracturen de fissuur bestaan. Dit zou veroorzaakt kunnen worden door abnormale botheling. Door de aanwezigheid van fibreus weefsel of dens trabeculair bot zou de botheling vertraagd of tegengehouden kunnen worden. Aangezien er geen volledige fusie of heling van de fissuurlijn mag verwacht worden, wordt de schroef constant belast, wat op termijn tot metaalmoeheid kan leiden met uiteindelijk het breken van de schroef tot gevolg. Indien dit gebeurt zal de patiënt terug pijn en kreupelheid vertonen (Figuur 9). In het ergste geval kan er zelfs een complete fractuur van de condylen optreden. Deze complicatie kan maanden tot jaren na de ingreep plaatsgrijpen. In dat geval is revisie noodzakelijk om de fractuur te behandelen of om de afgebroken schroef te vervangen (Marcellin-Little, 1994; Moores, 2006; Fitzpatrick et al., 2009).

Laser- en ultrageluidbehandeling, eventueel in combinatie met fourage, waarbij gaatjes geboord worden op plaatsen waar bot bloot is komen te liggen met als bedoeling littekenkraakbeen te creëren, zou ook een gunstig effect hebben (Bockstahler et al., 2004).

Toch zijn de afloop en prognose in de meeste gevallen niet zo ongunstig en de meeste honden recupereren zes tot acht weken na de ingreep. Ze hebben dan geen pijn meer, zijn niet kreupel en vertonen een goed functioneel herstel. De meeste patiënten met IOHC ontwikkelen op lange termijn echter milde osteoartrose. De symptomen zijn meestal mild en manifesteren zich door stijfheid na rust (Butterworth en Innes, 2001; Meyer-Lindenberg et al., 2002; Moores, 2006; Fitzpatrick et al., 2009, Favrilet al., 2014).

CONCLUSIE

Bij honden met elleboogkreupelheid moet incomplete ossificatie van de humeruscondylen zeker als differentiaaldiagnose worden overwogen. Indien radiografisch onderzoek geen eenduidige diagnose oplevert, is het uitvoeren van een CT-onderzoek zeker het overwegen waard. De prognose na het plaatsen van een compressieschroef is vrij gunstig.

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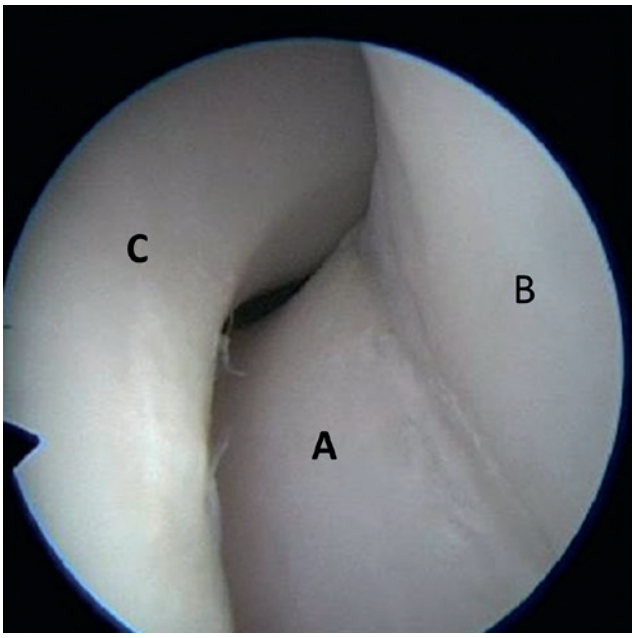

Figuur 7. Artroscopisch beeld van een linkerelleboog met IOHC. Er is een barst of fissuur in het gewrichtskraakbeen ter hoogte van de grens tussen de laterale en mediale humeruscondyl. A. Laterale humeruscondyl, B. mediale humeruscondyl, C. ulna met processus anconeus.

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Figuur 8. Een chirurgisch behandelde elleboog met IOHC, waarbij een compressieschroef werd geplaatst.

Figuur 9. Een complicatie opgetreden na het plaatsen van een compressieschroef. Door metaalmoeheid is er materiaalbreuk opgetreden. Een deel van de schroef is verwijderd en een resterend deel van de schroef is nog aanwezig.

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Over dieren

De kat is het enige dier dat erin geslaagd is de mens te domesticeren.

Marcel Mauss (Epinal 1872 - Parijs, 1950)
'Vader' van de Franse antropologie

Een hond heeft een baas, een kat heeft bedienden

Je 'hebt' geen kat, je deelt jouw huis met de kat

De Romeinse hondengrafvondsten van Tongeren

Roman dog burial in Tongeren (Belgium)

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SAMENVATTING

In Tongeren centrum werd bij een opgraving een graf van een grote hond gevonden uit de Romeinse periode. Ter hoogte van de mond lag een vaatwerkje als grafgift: een rond terra sigillata-schaaltje. Dit was of moest doorgaan voor het etensbakje van het dier. Door het afbreken van de randen wordt symbolisch aangegeven dat het bakje na de dood niet meer kon worden gebruikt. De symboliek van de etensbak met afgebroken randen kan ook geplaatst worden in een Keltische traditie waarbij mythische honden dagelijks stukjes van de volle maan afbeten en zo de maancyclus creëerden.

Deze vondst is uitzonderlijk, maar past in de zeer lange traditie van hondenbegrafenissen, met als vroegste vondst deze van Bonn-Oberkassel daterend van 14 200 jaar geleden en voortdurend tot in het heden. Ook nu nog geven mensen vaak voorwerpen mee bij een graf of urne van hun gezelschapsdier.

ABSTRACT

A skeleton of a large mature, probably male, dog was excavated in the city center of Tongeren, once a Roman city in Belgium. Together with the skeleton, a circular food bowl in terra sigillata pottery was discovered, positioned near the mouth of the dog, from which the upstanding edges had been removed. This was interpreted as a grave offering, representing symbolically that care was taken of the animal after death but that the bowl had become useless now. This habit of adding a circular bowl also fits in a Celtic mythical tradition, with dogs biting off pieces of the full moon and creating the moon cyclus. Circular bowls represent the moon and are related to the mythical dogs or wolves.

The archeological find is exceptional but fits into the long standing tradition of dog burials, starting 14 200 years ago with the Bonn Oberkassel dog and continuing into the present. Up till now, some owners still add goods to the resting place of their pets.

INLEIDING

In 2008 werd in Tongeren in het kader van de Anicius-onderzoeken - fase 2 en 3, ter hoogte van de Elfde-Novemberwal, een hondenskelet (V071 in S044) gevonden, met erbij ter hoogte van de snuit een terra sigillata-schaaltje met afgebroken randen (Panhuysen, 2009). Dit specimen is tentoongesteld in de afdeling 'Romeins' van het Gallo-Romeins Museum te Tongeren.

Het is de bedoeling van deze studie om deze begraven hond te beschrijven, de rapportage over deze vondst kritisch te bekijken, de representatie in de opstelling van het museum te bevragen en het geheel te kaderen in een ruimer geheel, zowel voor wat betreft de begrafenissen van honden doorheen de tijd, als voor wat de symbolische betekenis van deze begrafenis in Tongeren aangaat.

BESCHRIJVING VAN DE RESTEN

Het skelet van het begraven dier werd in werkput 4 gevonden op de bodem van een greppel. Het werd tijdens het uitgraven gedeeltelijk beschadigd door een graafmachine. Daardoor zijn enkele botten verloren gegaan. De arbeiders noemden de vondst Louise, wat laat veronderstellen dat ze dachten dat het een teef betrof.

Rapportage van de opgraving

In de beschrijving van de resten, zoals gepubliceerd in de rapportage van de opgraving, wordt gemeld dat het een dier betreft met een schofthoogte van 62 cm, wat overeenkomt met een Duitse herder. De groeiplaten/lijnen waren gesloten maar nog zichtbaar. Dit liet de archeologen toe te besluiten dat het een jong volwassene betreft van ongeveer anderhalf jaar oud dat volgens de rapportage nog aan het groeien was. Er wordt ook vermeld dat de houding van het skelet en de bijhorende voedingsschaal in aardewerk doen denken aan de sage van de maanbijtende wolf (zie verder), maar de houding van het skelet - belangrijk voor een dergelijk interpretatie - wordt niet beschreven. Tevens wordt genoteerd dat de rechterachterpoot ontbreekt.

Het schaaltje zelf bestaat quasi alleen uit een bodem met afgeslagen zijranden.

Herinterpretatie op basis van de foto's in de publicatie

De publicatie van de vondst bevat foto's van de opgraving en de berging van het skelet. Daaruit blijkt dat de linkerhelft van de schedel verbrijzeld is (door de graafmachine) want de schedel ligt in zijlig en dus lag de linkerschedelhelft bovenaan (Figuur 1). De ribben liggen deels links en deels rechts van de wervelkolom en het bekken horizontaal in buiklig (ventrale decubitus). De schedel en nekwervels zijn in rechterdecubitus gepositioneerd. De rechtervoorpoot ontbreekt, net als de rechterachterpoot en de ondervoet van het aanwezige linker voor- en achterbeen. Ook de staart ontbreekt.

Op deze foto's is ook te zien dat het een matuur dier betreft met gesloten groeiplaten dat dus niet meer groeit. Er zijn geen tekens van artrose in de heup, knie, schouder en de elleboog. Er is geen spondylose van de wervels. De vorm van de schedel en de grootte van het dier doen sterk vermoeden dat het een reu is, geen teef. Dat zou kunnen worden bevestigd door de aan- of afwezigheid van een penisbeen (baculum). Dat moet echter onder het bekken worden gezocht, wat niet mogelijk is in deze ingebedde toestand en met de positie van de hond op de buik. De hond werd dus begraven in rechter laterale decubitus in de kopregio en in ventrale decubitus vanaf de thorax.

Herinterpretatie op basis van het tentoongestelde skelet in het museum

In de expositiekast op de tentoonstelling in het Tongerse museum zijn schedel en schoteltje mooi uitgestald (Figuur 2). De schedel ligt nu echter horizontaal (ventrale decubitus) rustend bovenop de mandibula (deels geroteerd naar rechts) enquasi compleet, uitgezonderd wat minimale schade aan het rechterschedeldak. Dit is dus een heel ander zicht dan op de foto in Figuur 1. De tanden van de geëxposeerde schedel zijn behoorlijk aangetast door attritie en dus moet de schedel aan een dier van minstens zes jaar oud hebben toebehoord. Dat alles doet vermoeden dat de geëxposeerde schedel niet dezelfde is als deze op de foto. De tanden op de foto zelf zijn helaas niet te interpreteren wegens gebrek aan resolutie.

Het dier moet in ventrale decubitus in zijn graf gelegd zijn geweest, met de achterpoten naast zich en gebogen, met de knieën naar voor toe. De borstkas moet op het sternum hebben gerust zo dat na skeletvorming en verzakken, de ribben aan de twee kanten van de wervelkolom kwamen te liggen. De schedel moet ook sternaal hebben gelegen, allicht met de voorzijde van het viscerocranium in of op het schaaltje. Post mortem veranderingen (tafonomische processen) hebben hier gezorgd voor verzakking van de hals en schedel naar rechts. Deze rotatie naar laterale decubitus toe is logisch, omdat zulk een groot dier een diepe thorax heeft. Dat maakt dat er bij lijkvertering en druk door aarde, een zijkanteling is opgetreden.

Het schaaltje is uitgevoerd in terra sigillata (gestempeld aardewerk), type Dragendorff 36, (https://nl.wikipedia.org/wiki/Terra_sigillata). Het is glanzend rood-oranje vaatwerk typisch voor de Romeinse periode. De vorm van het schaaltje is op de gepu-

Figuur 1. De hondengrafvondst te Tongeren in 2008. De terra sigillata-schaalresten liggen links ter hoogte van de snuit (Afbeelding 112 uit Panhuysen (2009)).

Figuur 2. Het tentoongestelde hondenskelet in het Gallo-Romeins museum te Tongeren (Copyright foto: Gallo-Romeins Museum Tongeren).

bliceerde foto's niet direct te correleren met het geëxposeerde exemplaar dat mooi rond is en waarvan de opstaande randen netjes rondom afgebroken zijn (Figuur 2). Allicht is dit schaaltje gerestaureerd waardoor de ronde vorm hersteld werd, of werd er een ander schaaltje gebruikt voor de expositie.

SYMBOLIEK VAN HONDENBEGRAFENISSEN

Het opgravingsrapport verklaart de aanwezigheid van het schaaltje op basis van de sage van de maanbijtende wolven uit de Keltische traditie (https://nl.wikipedia.org/wiki/Kelten) (de Kelten zijn te dateren in de late ijzertijd van ongeveer 500 vóór Christus tot de Romeinse periode). Deze verhalen zouden in de Romeinse periode verder geleefd hebben, mogelijk in variante vormen.

De voedingsschaal staat in deze verhalen voor de symbolische maan (*Hollard*). De twee sagewolven uit de Keltische traditie (met de namen *Skoll* en *Hati*) zouden bij volle maan met geknabbel aan de maan starten en de maansikkel verklaren (de krimpende maan). Die dieren zouden dat verder doen tot ze helemaal verdwenen was tijdens de maanloze dagen van de maand. Daarna regenereerde de maan terug tot een volle maan en konden de wolven weer beginnen met gekluif (Guerber, 1863). Volgens een andere interpretatie worden enkel zon- en maansverduisteringen verklaard door dergelijke sagen.

Dit soort symboliek is maar een van de vele mogelijke verklaringen. Hoe te verklaren dat ook honden (of hondenresten of gecremeerde hondenbotten) werden begraven samen met mensen. Was dat als begeleider naar het hiernamaals? Als bewaker van het graf? Als compagnon om de meester te helpen bij de jacht in het hiernamaals?

Soms werden humane begrafenissen verrijkt met grafgiften (onder andere botten) of werden zulke giften zonder humane resten begraven, als symbolische offers, zoals bij het bouwen van een huis (bouwoffer) of tempel (Giffen, 1963; De Rijck, 2012). Toch was het botoffer bij bouw zeldzaam, want uit onderzoek in Vlaanderen betreffende de eerste drie eeuwen van onze tijdsrekening (Romeinse en Gallo-Romeinse periode), blijkt dat van alle bouwoffers er slechts 1% uit bot bestaat. Volledige skeletten zijn natuurlijk een erg kleine minderheid van alle botresten die ooit werden geofferd. Daarom is het zeer onwaarschijnlijk dat deze begraven hond een bouwoffer is.

De oudst bekende hondenbegrafenissen zijn deze uit het late Paleolithicum, voor de start van het Holoceen (11.700 jaar geleden). De meest bekende is zonder twijfel deze uit Bonn-Oberkassel in Duitsland: een dubbelgraf met hond (Street, 2002). Ook interessant zijn deze uit Predmosti, Tsjechië (Germonpré et al., 2012) waar Canidae (ook wolven) begraven werden met een rib in hun muil, allicht als een symbolische maaltijd voor het hiernamaals. In meer recente prehistorische perioden (mesoliticum en neoliticum) kwamen veel vaker begrafenissen van honden voor (Degerbøl, 1961a; Ewersen en Ramminger, 2010). Ze bleven bestaan in de bronstijd en ijzertijd (Polenz, 1975). Evenzo in de Egyptische (Bouvier-Closse, 2002), Griekse (Colonnelli en Mannino) en Romeinse tijd (Hollard, 1999) en tenslotte in de middeleeuwen, zoals bijvoorbeeld in de bootgraven van de Vikings (Prurnmel, 1992; Gräslund, 2004).

VERGELIJKBARE VONDSTEN

Een vergelijkbare vondst werd gedaan in 1965 in Mayen, nabij Koblenz, Duitsland (Grunewald, 2009). Het dier was ongeveer 45 cm groot en werd in zijlig gevonden in een individueel graf met een schaaltje uit terra sigillata met opstaande wanden (3,7 cm) die afgebroken waren maar aanwezig bij de bodem. De datering van de vondst is 40-70 n.C. Er waren geen snijsporen en geen pathologische afwijkingen te vinden op het hondenskelet. Ook hier werd een kommetje meegegeven in het graf en waren de boorden beschadigd, zij het veel minder extreem dan in het geval in Tongeren.

Er werden echter ook hondengraven gevonden waar enkel de fragmenten van de zijranden van de eetkom waren mee begraven, zonder het circulaire bodemdeel (Polenz, 1975), zoals er graven gevonden werden met vaatwerkscherven die zeker niet van een voedingskommetje afkomstig waren (Jorssen, 1963). Daarnaast kwam er nog een variant met als bijgifte een volledige en zeer grote kom die geen voedingbakje was (Chastel et al., 1995). En tenslotte worden ook soms begraven terra sigillata (type Dragendorff 36)-kommen gevonden, zoals in het graf in Tongeren, waarvan de randen rondom helemaal afgebroken zijn met enkel een overblijvend circulair bodemsegment, bijvoorbeeld te Velzeke en Sint-Denijs- Westrem, maar dan zonder hondenskelet (De Rijck, 2012).

Concluderend moet gesteld worden dat er een grote variabiliteit bestond in de wijze van het begraven van honden, met of zonder, al dan niet gemodificeerde voederschaaltjes.

DISCUSSIE

Het is verlokkelijk om de heidense verhalen van maanbijters als waarheid te aanvaarden: 'a good story seems a reasonable truth'. Maar er zijn ook andere denkpistes mogelijk. Zo zou de afgeslagen zijboord van de kom kunnen betekenen dat er na de dood van dit dier geen voedsel meer kan worden gegeten, dat het kommetje waardeloos was geworden, wat dan symbolisch uitgedrukt werd door de boorden te verwijderen.

Al bij al lijkt de variabiliteit binnen dit type van zeer zeldzame vondsten, te moeten leiden tot de vast-

stelling dat er geen logische en geen zekere conclusie kan getrokken worden. Zowel vloertjes van kommen als enkel randen ervan komen voor in hondengraven, zoals ook kommen die geen voedselbakjes waren, alsook scherven niet van voedselbakjes afkomstig. En natuurlijk waren er heel vaak ook geen bijgiften.

Waar zit het logische systeem dan? Wat is dan waar? Soms zoeken we symboliek omdat we dat prettig vinden, als mooie herinnering aan de sprookjes van onze kindertijd. Maar soms is de werkelijkheid veel eenvoudiger en saaier, minder romantisch en minder mooi. Mensen die nu afscheid nemen van hun hond, laten heel vaak dingen na bij het lijkje of de asse en de urn. Een speeltje, een balletje, iets wat gerelateerd is aan hun dier, iets wat emotie uitdrukt. Er waren geen pluche konijntjes of rubberen botsballen in de Romeinse periode, maar wel voederbakjes en dus waren die allicht datgene wat dezelfde emotie kon uitdrukken als de speeltuigjes van nu.

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Oproep

GEWIJZIGD STUDIEPROTOCOL: DARMGEZONDHEID EN IMMUNITEIT VAN DE HOND

In het kader van een studie rond darmgezondheid en immuniteit is het Labo Diervoeding van Universiteit Gent op zoek naar eigenaarshonden, die voldoen aan volgende criteria:

- Senior leeftijd (klein hondenras ≥8j, medium hondenras ≥7j, groot hondenras ≥6j, reuzenras ≥4j)
- de hond is momenteel gezond en krijgt geen medicatie
- de hond is het gewoon om korrels te eten
- de hond weegt meer dan 3kg
- de hond is niet extreem mager/zwaar (BCS 3-6/9)
- de hond heeft destijds de puppyvaccinaties gekregen en werd nooit eerder tegen **de ziekte van Lyme** gevaccineerd

Wat van de eigenaar verwacht wordt bij deelname aan deze studie:

- In de loop van de studie (4 maanden) 5x langskomen op de Faculteit Diergeneeskunde voor een gezondheidscontrole (incl. bloedname en onderzoek stoelgang). Bij de tweede en derde consultatie zal de hond eveneens worden gevaccineerd tegen de ziekte van Lyme.
- Gedurende de hele duur van de studie (4 maanden) de specifieke hondenkorrels van de studie voeren (exclusief en alleen deze hondenkorrels)
- De eetlust van de hond en de kwaliteit van zijn stoelgang 2x per week bijhouden in een dagboek + de hond 1x per week wegen gedurende een periode van 4 maanden

Wat de eigenaar mag verwachten bij deelname aan deze studie:

- 5x gratis gezondheidscontrole in de loop van de studie
- Gratis ontworming bij aanvang van de studie
- Gratis vaccinatie tegen de ziekte van Lyme
- Gratis hondenvoeding gedurende de hele studie (4 maanden)

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BEHANDELING VAN GIARDIA-INFECTIES BIJ DE HOND

VRAAG

"In de praktijk komen we meer en meer hardnekkige infecties met* Giardia *tegen. Vaak bij aangekochte dieren (broodfokkerijen). Klassieke behandelingen leveren niet het gewenste resultaat op. Wat is hiervan de oorzaak en hoe kan men dit aanpakken?"

ANTWOORD

Giardia-infecties bij de hond kunnen behandeld worden met fenbendazole (50 mg/kg p.o., drie tot vijf opeenvolgende dagen) of febantel 15 mg/kg in combinatie met pyrantel en praziquantel (drie opeenvolgende dagen) (http://www.esccap.eu/elements/uploads/ESCCAP%20RL6%20NL%20Darmprotozoen.pdf), Metronidazol wordt ook nog gebruikt (25 mg/kg oraal, 2 x per dag, gedurende 5 dagen), maar kan neurologische nevenwerkingen veroorzaken en zou kankerverwekkend zijn. Het gebruik van metronidazole voor de behandeling van giardiose is offlabel.

Er werd nog geen resistentie gerapporteerd van *Giardia* tegen deze producten bij de hond, hoewel resistentie tegen albendazole (Nash et al., 2001) en metronidazole (Lalle, 2010) wel beschreven werd bij de mens. Bij therapiefalen (i. e. de diarree geneest niet tijdens of onmiddellijk na de behandeling) moet rekening gehouden worden met co-infecties of een andere oorzaak van de diarree. Veel honden zijn immers asymptomatische dragers (Claerebout et al., 2009; Dupont et al., 2013) en kunnen ook positief testen voor *Giardia* bij andere oorzaken van diarree.

Zelfs na een ogenschijnlijk succesvolle behandeling hervallen honden met giardiose vaak na een korte periode van genezing. Enerzijds zijn de gebruikte producten niet 100% werkzaam en anderzijds treedt vaak herinfectie op door opname van cysten uit de omgeving. Daarom geven veel dierenartsen standaard een tweede behandelingskuur. Er zijn geen studies gepubliceerd over het ideale interval tussen de twee behandelingskuren. In de praktijk varieert dit meestal tussen vijf dagen en twee weken.

Hygiënische maatregelen zijn belangrijk om herinfectie vanuit de omgeving te verminderen en het risico op recidieven te verkleinen. Een behandeling gecombineerd met hygiënische maatregelen kan een *Giardia*-infectie onder controle houden (Saleh et al., 2016), hoewel hervallen zelfs bij doorgedreven maatregelen mogelijk blijft (Fiechter et al., 2012). Propere drink- en eetbakken gebruiken, het dagelijks wassen van de perianale regio en staartbasis van de hond om aangehechte cysten te verwijderen en het dagelijks verwijderen van de stoelgang (de uitgescheiden cysten zijn onmiddellijk infectieus) zijn maatregelen die de feco-orale transmissie van cysten kunnen verminderen. De verharde omgeving, bijvoorbeeld een terras, moet grondig gereinigd worden, zeker na defecatie. Daarna laat men het gereinigde oppervlak drogen, want *Giardia*-cysten zijn gevoelig voor uitdroging. Hitte is eveneens letaal voor *Giardia*-cysten. Hokken en buitenloop kunnen na het reinigen behandeld worden met stoomreiniging, gevolgd door drogen. De hondenmand kan gereinigd worden met een stofzuiger met stoomfunctie. In kennels kan eventueel desinfectie met quaternaire ammoniumpreparaten toegepast worden, maar enkel in open lucht of in een goed geventileerde ruimte (waarin geen dieren zitten!). Achteraf moet goed gespoeld worden, gevolgd door drogen. Bleekwater is hiervoor niet geschikt; *Giardia*-cysten zijn niet gevoelig voor chloor.

Nieuwe pups of kittens worden na aankomst in een nieuwe omgeving waar reeds andere huisdieren zijn, het beste in quarantaine geplaatst en getest (http://www.esccap.eu/elements/uploads/ESCCAP%20RL6%20NL%20Darmprotozoen.pdf).

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BOETE VOOR HET NIET-CASTREREN VAN EEN ADOPTIEHOND ?

VRAAG

"Een klant van mij heeft via een asiel in Wallonië een reu geadopteerd in 2016. In het adoptiecontract staat dat ze de hond moeten laten castreren. Er is medisch of gedragsmatig geen enkele reden om de hond te castreren en de eigenaars willen dit ook niet laten doen. Nu eist het asiel 500 euro boete.

Zijn zulke eisen rechtsgeldig?

ANTWOORD

In het adoptiecontract in casu staat een boetebeding, namelijk dat 500 euro boete moet betaald worden aan het asiel in geval van het niet-voldoen aan de opgelegde verplichting van castratie.

Het adoptiecontract is een rechtsgeldig contract, door beide partijen (asiel en adoptant) ondertekend. Boetebedingen zijn in beginsel geldige bedingen. Wel kan de rechter overmatige boetebedingen verminderen. De vraag of het bedrag in casu overmatig is, kan niet zomaar op algemene wijze worden beantwoord. Immers een dergelijke ingreep laten uitvoeren kost al snel meer dan 200 euro. Dus de beoordeling of het bedrag van 500 euro in casu al dan niet overmatig is, kan ook wat afhangen van het persoonlijk aanvoelen van de rechter.

Boetebedingen zijn ongeldig indien ze betrekking hebben op een verplichting die strijdig is met dwingende bepalingen van een wet. Een dergelijke wet is de Dierenwelzijnswet van 1986. Mocht daarin staan dat castratie bij honden verboden is, dan is samen met de verplichting om te castreren meteen ook het boetebeding dat de verplichting om te castreren kracht bijzet, ongeldig. Evenwel laat artikel 17bis van de Dierenwelzijnswet bepaalde ingrepen met het oog op de beperking van de voortplanting van dieren toe. Deze ingrepen moeten bij Koninklijk Besluit worden vastgesteld, en dit is gebeurd in het Koninklijk Besluit van 17 mei 2001. De castratie van honden staat op de lijst van toegelaten ingrepen. Aldus is het boetebeding in overeenstemming met de Dierenwelzijnswet.

In geval van een boetebeding kan degene ten voordele van wie dit is bedongen, kiezen ofwel voor de uitvoering in natura van de verplichting, ofwel voor de betaling van de boete. De twee zaken allebei vragen kan evenwel niet. Het asiel heeft in casu gekozen voor de betaling van de boete en dit houdt in dat indien de boete wordt betaald, de hond niet hoeft gecastreerd te worden. Indien de boete niet wordt betaald, heeft het asiel de keuze om voor de burgerlijke rechter ofwel te eisen dat de hond wordt gecastreerd, ofwel dat de boete alsnog wordt betaald.

Ook in Vlaanderen is het gebruikelijk dat de verplichting van castratie van een hond in een adoptiecontract wordt opgelegd. Daar wordt evenwel meestal aan toegevoegd "tenzij dit om medische redenen af te raden is voor het dier". Voor een dergelijke verklaring kan de dierenarts zorgen. Misschien dat het asiel hiermee ook zal instemmen in het geval van de vraagsteller, ofschoon het adoptiecontract in casu deze uitzondering niet voorziet. Overigens, in Vlaanderen ontbreekt doorgaans een boetebeding in een adoptiecontract.

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VETERINAIREN WAGEN ZICH AAN HUMANE GENEESKUNDE

Onder de vele duizenden zoogdiersoorten is de mens de enige waarvan dierenartsen moeten af blijven. Maar in het idyllische Ardeense dorpje Oucwègne beschreven in 'Mevrouw Verona daalt de heuvel af' van Dimitri Verhulst (uitgeverij Contact, 2006, p. 36-37) gaat het er enigszins anders aan toe. Een citaat:

"*Omdat de praktijk van de dichtstbijzijnde dokter zich in een naburig dorp bevond, ging zo goed als de voltallige bevolking van Oucwègne in geval van ziekte naar de dierenarts. Veel verschil kon er overigens niet schuilen tussen een varken en een mens wanneer je de anatomische prenten in de wachtkamer van Mme Lunette (de zwaar gebrilde dierenarts) mocht geloven, en wie de gelijkenissen weigerde te aanvaarden hoefde maar even op handen en voeten te gaan staan. Een zak met wat darmen en poten er aan, bij elkaar gehouden door een karkas en slijm. Deze kringloop van vreten en schijten voltrok zich in alle lijven, de hogere zowel als de lagere, de gaten dienden overal voor dezelfde smerigheid, en de luizen en de teken smaakten zelden het onderscheid. Hoe ze hun tijd tussen het neuken en het eten verdeelden durfde dan wel eens te variëren, ze waren beiden speelballen van bacteriën, kokken, bacillen en spirillen, met dezelfde klieren en dezelfde kankers, en wie de moed had om hierbij stil te staan, begon zich af te vragen waarom het esculapenschap zich had vertakt in een orde voor een beest en een orde voor de overige beesten.*"

Minder goed vergaat het veearts Richard in de grimmige novelle 'De Zwaardvis' (eerste druk 1987 als boekenweekgeschenk) van Hugo Claus. De compleet aan lager wal geraakte man, ongeveer de enige volwassene die met mededogen ten tonele gevoerd wordt, is destijds 'in de bak gevlogen' omdat hij vrouwen heeft 'geholpen' (abortus provocatus). "*Vrouwen die dachten dat ik als gediplomeerde iets kon doen aan hun miserie*" zo vertelt hij aan de jonge Maarten, de zwaardvis.

Onklopbaar blijft echter 'A Day at the Races' van de Marx Brothers, een film uit 1937. Dat zit zo in mekaar. De mooie Judy leidt een sanatorium. Om geld te verzamelen voor de afbetaling van haar lening doet ze een beroep op Dr. Z. Hugo Hackenbush. Die moet ervoor zorgen dat de rijke Emily Upjohn zo lang mogelijk in het sanatorium blijft. Zijn aanpak is nogal speciaal. Zo onderzoekt de dokter (Grouch Marx) patiënt Stuffy (Harpo, natuurlijk), neemt de pols en verklaart plechtig; 'Either this man is dead or my watch has stopped'. De rest van het personeel begint zich vragen stellen, als blijkt dat Hackenbush veearts is ...

Luc Devriese