

Oral pathological conditions of Felidae in captivity

Word count: <12.696>

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A dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Veterinary Medicine

Academic year: 2017 - 2018

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Acknowledgements

First of all, I wish to express my sincere gratitude towards my promotor, Dr. Hilde de Rooster, for her interest in my research proposal and allowing me to pursue my research goals by agreeing to be my promotor although she already had a busy schedule. Her guidance and firm but more than fair approach towards allowed me to unfold into a more critical mind which resulted into the end result of this thesis.

Also, I heartily thank Dr. Francis Vercammen and Annelies Michem from the KMDO (Koninklijke Maatschappij voor Dierkunde van Antwerpen), for their help and effort in persuading the participating EAZA zoos to cooperate in this study.

Furthermore, I would not forget to remember Leen Verhaert for providing her expertise and expressing her valuable opinion.

Obviously I would like to thank my parents, Tom Bollez and Nathalie Vandercruyssen, and my sister, Manon Bollez, for their support and encouragement throughout my entire studies of veterinary medicine and life in general.

Last but not least, I am thankful to my boyfriend, Jan Bearelle, for the constant support, motivation and his confidence in me.

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1 Abstract

This study was launched in the context of a final year thesis at Ghent University in association with Zoo Antwerp (BE), Planckendael (BE), Beekse Bergen (NL), GaiaZoo (NL), Dierenpark Amersfoort (NL) and Tierpark Chemnitz (DE). The study period stretched from December 2016 till March 2018. This is the first study - describing oral pathological conditions in captive Felidae - which has the approval and cooperation of EAZA zoos. Prospective data - during anesthesia or after euthanasia - as well as retrospective data, was collected from 18 captive Felidae enclosed in these EAZA zoos. Furthermore, skulls of Felidae - offered by EAZA zoos or encountered in private collections - were examined and the collected data was added to this study. The aim was mapping the current general oral health of captive Felidae. Hereby quantitatively describing the encountered oral pathological conditions - assessed in the study objects - and evaluating the effect of genetics, species, age, sex, diet, behavioral and environmental aspects as well as concomitant systemic diseases on the presence of oral pathological conditions to the extent possible. Studies in other mammalian species - domestic cats in particular - were assessed to deal with lacks of published research. Several traumatic and acquired non-traumatic oral pathological conditions - assessed in the 23 study objects - are described in this report. This pilot study may contribute to improving the knowledge of the general oral health of Felidae in captivity. Hopefully, new information about this matter will stimulate more in-depth investigations and may ultimately lead to the improvement of animal welfare and breeding programmes.

Samenvatting

Deze studie werd uitgevoerd in de context van een thesis aan de Universiteit Gent in associatie met de Zoo van Antwerpen (BE), Planckendael (BE), Beekse Bergen (NL), GaiaZoo (NL), Dierenpark Amersfoort (NL) en Tierpark Chemnitz (DE). De studieperiode reikte van december 2016 tot en met maart 2018. Dit is de eerste studie, handelend over orale pathologiën bij grote katachtigen in gevangenschap, die de goedkeuring én de medewerking verkrijgt van EAZA zoos. Retrospectieve data en Prospectieve data - verzameld gedurende anesthesie of na euthanasie - van 12 grote katten, gehuisvest in deze EAZA zoos, werden opgenomen in de studie. Ook werd er één schedel aangeboden door de Zoo van Antwerpen en meerdere schedels aangereikt door privé collecties. Deze werden onderzocht en de verzamelde data werd opgenomen in deze studie. Het doel was om de huidige orale gezondheidstoestand van grote katachtigen in gevangenschap in kaart te brengen. Hierbij werden orale pathologieën - gezien bij de studie objecten - kwantitatief beschreven en de effecten van genetica, diersoort, leeftijd, geslacht, gedrags- en omgevingsaspecten en systemische ziekten op orale pathologische condities bij grote katachtigen in gevangenschap geëvalueerd in de mate van het mogelijke. Studies bij andere zoogdiersoorten – gedomesticeerde katten in het bijzonder – werden geraadpleegd om hiaten op te vangen in de literatuur van grote katachtigen omtrent deze materie. Verschillende traumatische en verworven niet-traumatische orale pathologische condities – onderzocht in de 23 studie objecten – werden beschreven in deze studie. Deze pilot studie kan mogelijks bijdragen tot het verbeteren van de kennis over de algemene gezondheidstoestand van grote katachtigen in gevangenschap. Hopelijk zal nieuwe informatie omtrent deze materie meer diepgaand onderzoek stimuleren

2 Literature review

2.1 Introduction

Oral structures which are functionally and structurally normal are indispensable assets for mammals in order to survive and to reproduce. Carnivorous species in particular are dependent on their dentition. After all, their survival is all about catching, killing and eating prey animals, in which teeth play a leading role. Herbivores and omnivores, on the other hand, may come a long way using mainly oral structures other than teeth.

Metaphorically the oral cavity can be seen as the gateway to the mammal body. Some pathological conditions - determined by genetics, species, age, sex, diet, behavioral and environmental aspects as well as by concomitant systemic diseases - can unlock this gateway and possibly result in systemic disease. Oral pathological conditions can thus be seen as a severe violation of the fitness of mammals and carnivores in particular. Since certain parameters of animals in captivity are easy to trace, these specimens are obvious study objects.

This study focuses on captive big cats, species frequently kept in captive facilities open to the public. Only a small spectrum of literature about big cats is available but some extrapolations from domestic cats to big cats can be made.

2.2 Normal felid oral anatomy

Before elaborating on oral pathological conditions, minimal knowledge of functionally and structurally normal oral structures in big cats is indispensable. Consecutively, a brief summary will be given about both dentition and oral soft tissues - such as oral mucosa, lips (labia), cheeks (buccae), tongue (lingua glossa) with its papillae, the palate (pallatum) and salivary glands.

2.2.1 Dentition

Teeth are made out of 4 dental tissues of which 3 hard tissues (enamel, cementum and dentin) and 1 soft - or non-calcified - tissue (pulp). Dentin can be considered as the backbone of the tooth. The visible part - above the gum line - of the tooth (crown) is covered by enamel. Cementum covers the non-visible part - below the gum line - of the tooth (root). These two dentin covering hard tissues meet at the cervical portion of the tooth - the cement-enamel junction. The dentin aligns the pulp cavity which is filled up with pulp and consists of a pulp chamber - located in the crown - and a root canal - located in the root. The pulp contains nerves, vessels and connective tissue and is connected with periapical tissues via a number of foramina in the apex of the root (Reiter and Soltero-Rivera, 2014). Odontoblasts, located at the periphery of the pulp, continue to produce (secondary) dentin in vital teeth. This implies that permanent teeth of older cats have pulp cavities which are more narrow and dentinal walls which are thicker compared with permanent teeth of younger cats (Morse, 1991; Orsini and Hennet, 1992; Gracis, 2007; Park et al., 2014; Reiter and Soltero-Rivera, 2014).

Felidae are considered diphyodont, meaning the first set of teeth will be replaced by a second set as the animal ages. In domestic cats, primary or deciduous teeth erupt between 2 and 6 weeks of age. Eruption of the 30 secondary or permanent teeth starts at the age of 3 months and should be completed at the age of 6 months (Hale, 2005; Reiter and Soltero-Rivera, 2014). Unfortunately, tooth eruption and exfoliation in big cats is not described in the existing literature. All Felidae typically have a total of 26 deciduous and 30 permanent teeth: 6 incisors, 2 canines, 6 premolars and 2 molars in the maxilla and 6 incisors, 2 canines, 4 premolars and 2 molars in the mandibula (Table 1 and 2) (Miles and Grigson, 2003; Bellows, 2011). Congenital, developmental and traumatic abnormalities are occasionally described in all different felids (Verstraete et al., 1996a; Miles and Grigson, 2003). Hypodontia or the congenital absence of a tooth or teeth is less common in Felidae in comparison with other mammalian species. This might be explained by the fact that their dentition is already a lot more reduced compared to other species (Hall, 1940). Congenital tooth or teeth absence in domestic cats is most frequently seen in the premolar region (Verstraete et al., 1996b; Verstraete and Terpak, 1997; Verhaert and Van Wetter, 2004; Bellows, 2011; Rogers and Stern, 2017). According to limited literature, brachycephalic breeds would be predisposed (Kressin, 2009). The absence of the maxillary P2 is also regularly seen in a number of short-faced big cat species, such as the Lynx (*F. Lynx lynx* and *Lynx rufus*), Caracal (*F. caracal*), Cheetah (*Acinonyx jubatus*), manul (*Otocolobus manul*), leopard cat (*Felis Bengalensis*) and rusty-spotted cat (*F. rubiginosus*). However, in some big cats where the maxillary alveolar bone is rough between C and P3, P2 might be lost during life. Since missing rostral premolars is considered to be an individual anomaly, a minimum of 26 permanent teeth is assumed (Kurtén, 1963; Ewer, 1973; Glass and Todd, 1977; Verstraete et al., 1996a; Gomerčić et al., 2009; Miles and Grigson, 2003; Geraads, 2014; Leemans, 2015; Aghashani et al., 2016).

Table 1: Dental formula of the feline deciduous dentition.

Maxilla								
i1	i2	i3	c1	p2	p3	p4	x2 = 14	= 26
i1	i2	i3	c1		p3	p4	x2 = + 12	
Mandibula								

Table 2: Dental formula of the feline permanent dentition.

Maxilla									
I1	I2	I3	C1	P2	P3	P4	M1	x2 = 16	= 30
I1	I2	I3	C1		P3	P4	M1	x2 = + 14	
Mandibula									

Cats use their 12 incisors to groom and to hold onto prey, 4 canines to penetrate and grasp prey and 10 premolars together with 4 molars to grind prey (Reiter and Soltero-Rivera, 2014). The first lower (mandibular) molar and the fourth upper (maxillary) premolar are the carnassial, sectorial or shearing teeth which are adapted to allow shearing (instead of tearing) of prey flesh in order to permit more efficient meat consumption (Orsini and Hennet, 1992; Gracis, 2007; Reiter and Soltero-Rivera, 2014).

Hypothetically, interspecific competition for prey and/or ethological interactions (e.g. canine display for sexual selection or use during fights inter- and intraspecifically) may have been the mobilizing factor for the existing size ratio of canines in all carnivorous species and thus also in felids (Dayan et al., 1990). Indeed, canine tooth assets (size and strength), but also body weight and skull morphology - such as condylobasal-length and zygomatic-breadth (the distance between both zygomatic branches) are proven to be associated with prey size (Rosenzweig, 1966; Kleiman and Eisenberg, 1973; Radinsky, 1981; Gittleman, 1985; Valkenburgh and Ruff, 1987; Dayan et al., 1990; Seidensticker and McDougal, 1993). Large cats (>25kg) with big canines hunt prey larger than or equal to their own size whereas small cats (<15kg) with little canines catch prey smaller than their own size. As an exception to this, some larger 'small cats' (15-25kg) also take prey equal to and larger than their own size (Cuff et al., 2015). Since environmental variables (e.g. diet, season and age) can have a big influence on body weight and skull morphology, measurements of weight and skull are assumed to correlated less to prey size than canine tooth assets (Valkenburgh and Ruff, 1987; Valkenburgh, 1988; Dayan et al., 1990; Seidensticker and McDougal, 1993). Also, skull morphology is determined by a number of other defining factors (e.g. brain mass, senses, muscle mass...) besides prey size (Moore, 1981).

2.2.2 Oral soft tissues

The oral cavity, lined by its soft tissues, is the first barrier towards external pathological agents (Harley et al., 2003). Oral mucosa consists of partly cornified, stratified squamous epithelium. The underlying submucosa consists of connective tissue and mixed glands. Near the incisive bone and alveolar processes of the maxilla and mandibula, the oral mucosa is modified into the gingiva.

Lips (*labia oris*) and cheeks (*buccae*) are made out of skin, a muscular layer and oral mucosae and are used for the prehension and retention of food, communication and suckling in neonati. The tongue (*lingua glossa*) - subdivided into an apex (*apex linguae*), a body (*corpus linguae*) and a root (*radix linguae*) - basically consists of muscular tissue wrapped up in mucosa and is used for the uptake of water, processing food and swallowing. A median groove, the *sulcus medianus*, makes a longitudinal mark in the dorsal lingual body surface. The *frenulum*, a mucosal fold, connects the ventral side of the tongue to the bottom of the oral cavity. A variety of papillae occupies the lingual body. In *Felidae*, filiform papillae - present on the entire dorsal lingual body surface - are the most remarkable. Other mechanical papillae such as fungiform and vallate papillae are distributed dorsally on the lingual body as well. Foliate papillae, on the other hand, are not presented on the felid tongue (Emura et al., 2014). The palate (*pallatum*) - composed of a rostral hard palate (*palatum durum*) and a caudal soft palate (*pallatum molle*) and covered with transverse mucosal ridges - separates the oral from the nasal cavity. Secretion of the salivary glands - parotis, mandibular, zygomaticus, buccal and sublingual - emerges at different locations in the oral cavity, allowing saliva to start food digestion (König and Bragulla, 2007).

2.3 Correlation between oral health and systemic conditions

The impact of oral health on systemic conditions is a well-described subject in the existing literature in human medicine. Supportive evidence for the importance of oral health in various internal infectious diseases is provided. Correlations were found between periodontal diseases and cardiovascular diseases such as endocarditis, myocarditis and atherosclerosis (Palank et al., 1979; Süzük et al., 2016; Mahalakshmi et al., 2017). Some experimental studies in mice present the same outcome (Ashigaki et al., 2013; Chukkapalli et al., 2015). Respiratory diseases have also been linked to periodontal diseases (Muthu et al., 2016). Furthermore, a wide range of other clinically important systemic diseases have been associated with periodontal diseases and overall oral health in human medicine such as chronic kidney disease, diabetes, liver cirrhosis, pancreatic cancer (Glickman et al., 2011; Lalla and Papapanou, 2011; Farrell et al., 2012; Greene et al., 2014; Gronkjaer, 2015). Maternal periodontitis may play an important role in adverse pregnancy outcomes (Offenbacher et al., 1998; Ren and Du, 2017). Results of prospective studies in dogs also indicates that periodontal disease can lead to systemic effects (DeBowes et al., 1996; Glickman et al., 2011; Rawlinson et al., 2011). Limited literature suggests that periodontal disease could be a risk factor for CKD in domestic cats (Greene et al., 2014). Other extended data is not available in domestic cats, not to mention in big cats, but it is very likely that the situation is similar in all mammal species. Cats, like humans, are very frequently affected by oral pathological conditions (Adler et al., 2016). Presumably, the situation in big cats is comparable, although poorly described until now.

2.4 Frequently seen oral pathological conditions in Felidae

Periodontal disease is the most common oral pathological condition in domestic cats (Perry and Tutt, 2015). Gingivitis, caused by plaque bacteria on the surface of the teeth, is the initial stage of periodontal disease and can be reversed by consistent daily thorough dental care (e.g. tooth brushing) (Perry and Tutt, 2015). The later stage of periodontitis is characterized by gingival recession, periodontal pocket formation, or both. Irreversible periodontal bone loss will occur in the end stage although its progression can still be arrested by proper treatment. This disease will ultimately result in tooth loss (Niemić, 2008; Perry and Tutt, 2015). Periodontal disease in big cats is barely described in the existing literature. It has been suggested that wild Felidae species are less likely to develop these issues due to their strict diet containing fresh meat on the bone. Big cats in captivity are also fed a strict carnivorous diet. Therefore, it is hypothesized that the majority of captive Felidae are also periodontal disease-free (Miles and Grigson, 2003).

Tooth resorption is another oral pathological condition frequently diagnosed in domestic cats. The exact aetiology is unknown but this condition is characterized by enamel and dentin resorption of the affected teeth due to the activation of odontoclasts (Niemić and FAVD, 2012). A study in a colony of 109 cats, housed at the Royal Canin Centre, found a significantly higher prevalence of tooth resorption in pure-breed cats compared to mixed-breed cats (Girard et al., 2008). In contrast to periodontal disease, tooth resorption is well described in several species of big cats (Kertesz, 1993; Berger et al., 1996; Roux et al., 2009; Pettersson, 2010; Aghashani et al., 2016; Aghashani et al., 2017; Steenkamp et al., 2018).

2.5 Dietary importance and role in oral pathologies of Felidae

Just like domestic cats, big cats are true carnivores and rely on the intake of prey tissue to receive their nutritional needs. Taurine, arginine, arachidonic acid, vitamin A (as retinol), vitamin D and niacin are essential in a healthy felid diet (MacDonald et al., 1984; Tilson et al., 1994; Morris, 2002). Certain endogenous synthetic metabolic pathways in domestic cats became redundant and energy inefficient, considering the abundant presence of amino acids (Taurine, arginine and their precursors), vitamin D, niacin and animal fat (Arachidonic acid and its precursors) and paradoxically the negligible concentrations of carotenoids (Precursor for vitamin A) in the tissues of their prey (Rivers et al., 1975; Knopf et al., 1978; Sinclair et al., 1979; Pawlosky et al., 1994; Morris, 1999b; Kirk et al., 2000; Morris, 2002;). This probably became the decisive evolutionary factor for relying almost entirely on the dietary income of these essential nutrients (Zoran, 2002). Consequently, the metabolism of domestic cats has adapted in such a way to use protein and fat as preferred energy sources (Zoran, 2002). The existing literature suggests that an extrapolation concerning these nutritional needs can be made from domestic cats to all felid species (Davidson et al., 1986; Howard et al., 1987; Burton et al., 1988; Crissey et al., 1997; Bechert et al., 2002; Lange et al., 2017).

Apart from the chemical dietary characteristics, emphasizing physical (consistency and moisture content) and psychological characteristics of the diet is necessary. It seems that it is the physical rather than the chemical character which is held responsible for the development of periodontal disease (Miles and Grigson, 2003). Soft texture diets do not provide sufficient abrasive activity on the teeth surface. Feeding fresh meat on the bone contributes to better gingival and periodontal health and stimulates natural feeding behavior, which enhances the activity levels and pleasure in food

consumption. Studies with captive Felidae found improvement in appetite and body condition when fasted one or two days a week, implying increased meal sizes per serving (Traylor-Holzer, 2010). Dietary consistency, moisture content as well as feeding interval are predominant - alongside dietary chemical composition - when it comes to physical and psychological health of Felidae in captivity (Haberstroh et al., 1984; MacDonald et al., 1984; Tilson et al., 1994; Felicetti et al., 2008; Szokalski et al., 2012).

2.6 Genetic induced skull conformations

Cat domestication took place about 10,000 – 4,000 years ago. Wildcats (*Felis silvestris*) roamed all over the Old World (Europe, Africa and Asia). According to current taxonomy, there are four wild subspecies, *F. s. silvestris*, *F. s. lybica*, *F. s. ornata* and *F. s. cafra*. Studies of mitochondrial DNA from domestic cats and modern wildcats indicate that ancient Near Eastern and Egyptian cat lineages of *F. s. lybica* were the maternal ancestors of the domestic cats we know today. Food storage and waste around human colonies brought about the presence of rodents, encouraging cats to live nearby. Within the last 200 years, the artificial selection of domestic cats took a giant leap to produce a diversity of cat breeds (Driscoll et al., 2009; Gandolfi and Alhaddad, 2015). There are about 40-55 designated breeds of domestic cats around the world (Morris, 1999a; Gandolfi and Alhaddad, 2015). Selecting specific physical characteristics limits the number of cats that can be used as foundation stock, which implies inbreeding and reduced genetic variability. Fixing the desirable trait (e.g. brachycephaly, curly hair coat, hairless, ventral ear fold, rostral curled pinnae, short-limb, short-tail ...) inevitably goes hand in hand with fixing other unrelated traits and unfortunately also undesirable traits (e.g. polycystic kidney disease, hypertrophic cardio myopathy...) (MacDonald et al., 1984; Malik et al., 1999; Gandolfi et al., 2010; Gandolfi and Alhaddad, 2015; Lyons, 2015; Pollard et al., 2015).

Brachycephaly was desired and pursued in some breeding programmes creating extreme short-faced breeds such as Persians and Exotic shorthairs. Unfortunately, high degrees of brachycephaly in domestic cats are correlated with malformations of the skull cavity, brain and facial bones as well as dental abnormalities (Schmidt et al., 2017). Studies in brachycephalic domestic cats demonstrate how severe the distorted conformation of their skulls has become (Malik et al., 2009; Schlueter et al., 2009; Schmidt et al., 2017). The brain is stuffed into a brain cavity of the wrong size. Supposedly, there will soon be cases of Chiari-like malformations and syringomyelia in brachycephalic cats, just like in Cavalier King Charles spaniels. Due to the shortening of the jaw, there is less space in the oral cavity. Severe overcrowding and rotation of the teeth influence the eruption process. Teeth may only partially erupt or even fail to do so and remain embedded underneath the gingiva (Kressin, 2009). Proper mastication is compromised because the teeth erupt at such bizarre angles. Food will accumulate between the teeth, promoting plaque, calculus, gingivitis, and subsequently periodontal disease (Malik et al., 2009; Schlueter et al., 2009). As these studies provide the evidence that brachycephaly can affect animal welfare, selection for extreme forms of brachycephaly in certain cat breeds should be reconsidered (Schmidt et al., 2017). Cranial malformations comparable with these in brachycephalic cat breeds have been reported in related white lions and cheetahs in captivity (Scaglione et al., 2010; Steenkamp et al., 2018). In contrast to short-faced domestic cats, this trait was not at all desired in these Felidae species. On top of that, this trait also came along with other undesirable traits just like in domestic brachycephalic breeds. Several studies in captive lions already describe Chiari-like malformations (Baker and Lyon, 1977; Papendick et al., 1995; Shamir et al., 1998; McCain et al., 2008).

Zoological parks breed a diversity of mammal species and either due to a lack of available animals to pair or because there used to be a lack of concern about the effects of inbreeding, zoos have often extensively inbred their stocks. Therefore, the breeding records of zoos potentially offer plenty of data on the effects of inbreeding (Thornhill, 1993). "Inbreeding depression" is a decrease in the value of a trait as a result of inbreeding. This phenomenon can be explained by the alteration of the genotype frequencies, namely the number of homozygotes increases. Due to this, the effects of harmful recessive alleles will be more reflected (Shields, 1987; Scaglione et al., 2010;). Mainly metric traits indirectly associated with fitness (e.g.: birth weight, craniometric measurements...) and reproductive traits (e.g.: fecundity, perinatal viability...) are involved when it comes to inbreeding depression (Crnokrak and Roff, 1999; Xu et al., 2007; Scaglione et al., 2010; Godoy et al., 2016). Malformations involving structures of the head (jaw, tongue, throat, teeth, and cranial bones) are reported in related white lions (Scaglione et al., 2010).

The European Association of Zoos and Aquaria (EAZA), which is currently the largest professional zoo and aquarium association in the world, was founded in 1992. Its mission statement is to facilitate and coordinate cooperation and communication between its members of the European zoo and aquarium community in order to achieve its goals of education, research and conservation. EAZA never claimed that the presence of animals in zoos and aquaria will make in situ action in the wild redundant. However, EAZA is convinced that the zoo and aquarium community, together with its visitors, can contribute to more knowledge about animals - their way of living, interactions and habitat - and help finance field conservation projects. Also, zoos and aquaria can serve as a database for genetic diversity of animal species in the wild and that way safeguard the future. The genetic diversity of the founders of zoo and aquarium animal populations is being retained as much as possible. In other words, evolution is practically stopped in the captive populations. Founders, belonging to the same taxon and presumably unrelated, determine the quality of breeding programmes. As opposed to wild populations, populations in captivity are relatively small and show little gene flow between subpopulations - different enclosures and institutions - which makes captive populations very vulnerable for the loss of genetic diversity, promoting inbreeding and "inbreeding depression".

Genetic diversity can - among other methods - be measured through the use of specialized computer models, based on pedigree information. If more genetic diversity is desired, a bigger population is required. These computer models can calculate the minimum population size needed in order to maintain 100% of the genetic diversity. The result is always a population which is simply too big to be kept in zoos. However, tolerating a little loss of genetic diversity (10%) results in the need of a smaller population which can be kept in zoos. 90% genetic diversity corresponds with a common inbreeding level of 10% or an inbreeding coefficient of 0,10 which is considered acceptable. EAZA has two different levels of breeding programmes at the moment: the European Endangered species Programme (EEP) and the European Studbook (ESB). Tasks of the studbook keeper, responsible for a certain ESB, include coordinating breeding and transfers as well as collecting and analyzing data such as births, deaths and other relevant information on the species - which allows to judge whether a more intensive breeding programme is required for this species in particular. If this is in fact the case, the studbook keeper can file a request to the authorized EAZA institution, demanding that a more intensive breeding programme - suggesting an EEP programme - may be required for this particular species. The coordinator of an EEP programme, assisted by a species committee, is besides the studbook also responsible for executing demographic and genetic analyses as well as producing a plan for the future management of the species in the European zoo and aquarium community.

2.7 Research problem and study objective

The existing literature taken into account, investigating oral pathology of Felidae in captivity could be very interesting. Oral pathological conditions are potential contributing factors for a wide range of clinically important systemic diseases. Currently, no summarizing literature describing the effect of different parameters - genetics, species, age, sex, diet, behavioral and environmental aspects as well as concomitant systemic diseases - on the presence of oral pathological conditions in captive Felidae exists.

The aim was mapping the current general oral health of captive Felidae. Hereby quantitatively describing the encountered oral pathological conditions - assessed in the study objects - and evaluating the effect of genetics, species, age, sex, diet, behavioral and environmental aspects as well as concomitant systemic diseases on the presence of oral pathological conditions to the extent possible. Dedicated forms were prepared with the aim of following a strict research protocol in order to collect data in a standardized way.

3 Materials and methods

This study was launched in the context of a final year thesis at Ghent University in association with Zoo Antwerp (BE), Planckendael (BE), Beekse Bergen (NL), GaiaZoo (NL), Dierenpark Amersfoort (NL) and Tierpark Chemnitz (DE). All of the organisations are EAZA members, housing big cats in captivity.

3.1 Data collecting

The approach was to collect both retrospective and prospective data of Felidae (including but not limited to lions, tigers, leopards, jaguars, cheetahs, and snow leopard) in captivity. Prospective data was collected when the animal underwent anesthesia or euthanasia in an EAZA zoo. Also skulls of Felidae - offered by EAZA zoos or encountered in private collections - were examined and the collected data was added to this study. Dedicated forms were prepared in order to collect all the desired retrospective data - signalment, zoo of residence or private collection, medical history, dietary properties and pedigree information - in a standardized way (Supplement 1). From now on, the group of big cats that underwent anesthesia or euthanasia in an EAZA zoo will be referred to as the AN/EU group. Also, the term SK group will be used to refer to the group of skulls offered by zoos or encountered in private collections.

Signalment included species (lion, tiger, leopard, jaguar, cheetah, snow leopard...), age (exact or estimated), gender (male or female and neutered or not) and body weight (exact or estimated) if the information was accessible. Current zoo of residence of the anesthetized or euthanized animal at the time of data collecting was noted. In case of a skull, the origin (offered by EAZA zoos or encountered in private collection) was mentioned. When accessible and applicable, medical history was also taken into account. Dietary properties such as consistency and moisture content (soft and humid – pure raw meat, intermediate – combination of bone with raw meat – hard and dry – pure bone, purely theoretical) and feeding interval (days fed/week) were obtained. Supplements to the diet were also noted. Efforts were made in order to collect pedigree information of each study object included in this study with the intention to calculate the coefficient of inbreeding of each individual big cat using Wright's formula (Supplement 1).

Examination of the study objects included making notes on the dental formula (present on the dedicated forms) and checking the presence of oral congenital or acquired traumatic and/or non-traumatic pathological conditions concerning quantitative and/or qualitative conformation disorders of the cranium, jaw, teeth and - in case of a big cat belonging to the AN/EU group - oral soft tissues. All teeth were referred to by a number according to the modified Triadan system (Floyd, 1991; Crossley, 2002) (Supplement 2).

3.2 Descriptive statistics

Analyzing the collected data, in order to quantitatively describe the encountered oral pathological conditions - assessed in the study objects - and identify a possible correlation between oral pathological conditions and genetics, species, age, sex, diet, behavioral and environmental aspects as well as by concomitant systemic diseases to the extent possible.

4 Results

This study includes the data of captive big cats enclosed in Zoo Antwerp (BE), Planckendael (BE), Beekse Bergen (NL), GaiaZoo (NL), Dierenpark Amersfoort (NL) and Tierpark Chemnitz (DE). Furthermore, captive bred big cat skulls from zoo and private collections were examined and the collected data was added to this study. During the study period from December 2016 till March 2018, data was collected of a total of 23 study objects.

Since different EAZA zoos participated in this study, the artificial habitats of these animals can differ. Therefore, this geological parameter was taken into account. The AN/EU group contained two big cats from Zoo Antwerp (2/18), five big cats from Planckendael (5/18), seven big cats from Beekse Bergen (7/18), three big cats from Gaia Zoo (3/18) and lastly one big cat from Tierpark Chemnitz (1/18). Those three big cats from Gaia Zoo had undergone anesthesia for transport towards Dierenpark Amersfoort. As far as the SK group is concerned, one skull was presented by Zoo Antwerp (1/5). Since the other four skulls belong to a private collection, the geological parameter cannot be tracked.

A total of eighteen study objectives belong to the AN/EU group (18/23). Fifteen of them underwent anesthesia (15/18) and three of them were euthanized (3/18). The SK group consists of five skulls (5/23) of which only one was offered by zoo Antwerp (1/5) and the others encountered in a private collection (4/5).

Four different species of big cats enrolled in this study: *Panthera leo* (both *leo leo* and *leo persica*, but no further distinction was made between these subspecies), *Panthera tigris*, *Panthera pardus* and *Acinonyx jubatus*. Among the AN/EU - group, the following species were represented: *Panthera leo* (15/18), *Panthera tigris* (1/18) and *Acinonyx jubatus* (2/18). The SK group contained the following species: *Panthera leo* (3/5), *Panthera pardus* (2/5).

Information about the exact age was accessible of every big cat enclosed in an EAZA zoo that had undergone anesthesia or euthanasia. Also, the exact age at death of the skull offered by zoo Antwerp was available. The age at death of all the other study objectives belonging to the SK group was unknown and could not be estimated. Different age categories were represented in this study. The following subdivisions were made: “<2”, “2 to 5”, “6 to 10”, “11 to 20” and “> 20”. In the AN/EU group, three big cats belong to the “<2” age category (3/18), six big cats are part of the “2 to 5” age category (6/18), two big cats are included in the “6 to 10” age category (2/18) and seven big cats form part of the “1 to 20” age category (7/18). Not one big cat fitted in the “>20” age category. As for the SK group, only one of them is included in the “11 to 20” age category whereas the age of the other four skulls is unknown.

In this study, both sexes were represented. The AN/EU group consists of fewer males (4/18) than females (14/18). As for the SK group, two females (2/5) were recorded while the sex of the remaining skulls (3/5) was unknown. No specimen - whose sex is known - was neutered. In the context of birth control, female big cats receive an implant in the EAZA zoos that participated in this study.

Weight was only recorded in some of the big cats encountered in Zoo Antwerp and Planckendael. In all other cases weight was unknown and could not be estimated accurately. Therefore, the parameter “weight” was no longer taken into account in this study.

A clear brief summary of each study object included in this study with its different parameters of interest can be found in the table below (Table 3).

Table 3: Overview of each specimen included in this study with its different parameters of interest.

NAME	n°	CAPTIVE FACILITY	GROUP	SPECIES	AGE (CATEGORY)	SEX
Arlen	1	Beekse Bergen	<u>AN</u> /EU	<i>Panthera leo</i>	8 (6 to 10)	V
Cocky	2	Beekse Bergen	<u>AN</u> /EU	<i>Panthera leo</i>	4 (2 to 5)	V
Evy	3	Beekse Bergen	<u>AN</u> /EU	<i>Panthera leo</i>	4 (2 - 5)	V
Marga	4	Beekse Bergen	<u>AN</u> /EU	<i>Panthera leo</i>	13 (11 - 20)	V
Petra	5	Beekse Bergen	<u>AN</u> /EU	<i>Panthera leo</i>	11 (11 - 20)	V
Steffie	6	Beekse Bergen	<u>AN</u> /EU	<i>Panthera leo</i>	5 (2 - 5)	V
Zulu	7	Beekse Bergen	<u>AN</u> / <u>EU</u>	<i>Acinonyx jubatus</i>	14 (11 - 20)	V
Lewa	8	Gaia Zoo	<u>AN</u> /EU	<i>Panthera leo</i>	1 (<2)	V
Sabi	9	Gaia Zoo	<u>AN</u> /EU	<i>Panthera leo</i>	1 (<2)	V
Zaila	10	Gaia Zoo	<u>AN</u> /EU	<i>Panthera leo</i>	1 (<2)	V
Koyla	11	Planckendael	<u>AN</u> /EU	<i>Panthera leo</i>	15 (11 - 20)	V
Lorena	12	Planckendael	<u>AN</u> /EU	<i>Panthera leo</i>	8 (6 - 10)	V
Raman	13	Planckendael	<u>AN</u> /EU	<i>Panthera leo</i>	2 (2 - 5)	M
Rani	14	Planckendael	<u>AN</u> /EU	<i>Panthera leo</i>	2 (2 - 5)	V
Ravi	15	Planckendael	<u>AN</u> /EU	<i>Panthera leo</i>	2 (2 - 5)	M
Sam	16	Tierpark Chemnitz	<u>AN</u> / <u>EU</u>	<i>Panthera tigris</i>	19 (11 - 20)	M
Caitlin	17	Zoo Antwerp	<u>AN</u> /EU	<i>Panthera leo</i>	16 (11 - 20)	V
Poko	18	Zoo Antwerp	<u>AN</u> / <u>EU</u>	<i>Acinonyx jubatus</i>	16 (11 - 20)	M

Maouli	19	Zoo Antwerp	SK	<i>Panthera leo</i>	20 (11 - 20)	V
Lion v	20	Private collection	SK	<i>Panthera leo</i>	Unknown	Unknown
Lion w	21	Private collection	SK	<i>Panthera leo</i>	Unknown	Unknown
Panther a	22	Private collection	SK	<i>Panthera pardus</i>	Unknown	Unknown
Panther s	23	Private collection	SK	<i>Panthera pardus</i>	Unknown	Unknown

Information about nutrition could only be obtained of study objectives belonging to the AN/EU group encountered in EAZA zoos and the one sole skull presented by zoo Antwerp. In every EAZA zoo that participated in this study, the animals are fed 4 to 5 days a week and dietary properties classify as “intermediate”. Also, in all of these EAZA zoos, “Carnizoo” (Kasper Faunafood) is supplemented to the enclosed big cats.

Medical history was unknown or not accessible in most study objectives. In each case of an accessible medical history, no oral pathologies were described. Elaborating on this subject would take us too far within the context of this study. A small detail worth mentioning is the fact that tooth exfoliation had taken place in three of the study objects - one year of age - at the time of examination (n°8,9,10). Deciduous canines were still visible next to the erupted permanent teeth (Picture 1-3). All other teeth were already completely exfoliated.



Picture 1: Tooth exfoliation (n°9) Picture 2: Tooth exfoliation (n°9) Picture 3: Tooth exfoliation (n°8)

It was not possible to collect sufficient pedigree information about each different study object in order to properly analyze pedigree data. Therefore, no results about this matter can be displayed.

Congenital abnormalities - such as mandibular mesiocclusion, mandibular distocclusion, palatoschisis, hyperdontia, hypodontia and microdontia - were not found in any of the 23 study objects.

Traumatic abnormalities such as tooth fractures, tooth attrition or abrasion and tooth absence (presumably tooth avulsion due to trauma) were sporadically seen (Table 4). Four (4/18) specimens of the AN/EU group had a fracture of a canine tooth, hereby exposing the pulp cavity (Picture 4). More specifically: tooth 204 (n°1), tooth 104 (n°4,11) and tooth 404 (n°12). Tooth fractures were not observed in the SK group. Tooth attrition or abrasion was present respectively in five (5/18) and three (3/5) specimens of the AN/EU group and SK group (Picture 5, 6 and 7). In detail: teeth 101-103 (n°4,16,22), teeth 201-203 (n°4,16), teeth 301-303 and 401-403 (n°16), tooth 104 (n°18), tooth 204 (n°18,23), tooth 304 (n°16,17,22), tooth 404 (n°16,23), tooth 107 and 207 (n°4,5), tooth 307 and 407 (n°5) and the carnassial teeth 108, 208, 309 and 409 (n°20). Both cheetahs (2/18) of the AN/EU group and the skulls of both panthers (2/5) were seen with tooth absence - tooth avulsion (Picture 8). One cheetah showed absence of teeth 101 and 403 (n°7) and the other missed the following teeth: 101, 102, 201-203, 301, 401 and 402 (n°18). In one of both panthers teeth 104 and 204 (n°22) were absent (Picture 8). The other panther showed absence of the following teeth: 304, 101, 102, 201, 202, 301-303 and 401-403 (n°23). Mucosal ulcerations could be found in eight (8/18) specimens of the AN/EU group (n°2,3,5,11,13,15,16,17) (Picture 9). The presence of mucosal ulcerations could obviously not be assessed in the skulls.



Picture 4: Complicated crown fracture of the upper left canine (n°1).



Picture 5: Tooth Abrasion of both lower canine teeth (n°16).



Picture 6: Tooth attrition of the right lower carnassial tooth (n°20).



Picture 7: Tooth Abrasion of the right lower canine tooth (n°23).



Picture 8: Tooth avulsion of the incisor teeth (n°18).



Picture 9: Mucosal ulcerations of the hard palate (n°15).

Table 4: Traumatic abnormalities

	Tooth fracture	Tooth attrition/ abrasion	Tooth absence (avulsion)	Mucosal ulcerations
n°1	x			
n°2				x
n°3				x
n°4	x	x		
n°5		x		x
n°6				
n°7			x	
n°8				
n°9				
n°10				
n°11	x			x
n°12	x			
n°13				x
n°14				
n°15				x
n°16		x		x
n°17		x		x
n°18		x	x	
n°19				-
n°20				-
n°21		x		-
n°22		x	x	-
n°23		x	x	-
Total	4 (/23)	8 (/23)	4 (/23)	8 (/23)
AN/EU	4 (/18)	5 (/18)	2 (/18)	8 (/18)
SK	0 (/5)	3 (/5)	2 (/5)	-

Acquired non-traumatic oral pathological conditions were frequently encountered (Table 5). Discolouration of teeth was only seen in a sole lion skull (*n*°20) (Picture 10). Tooth resorption was not detected in any of the 23 study objects. Obvious plaque was present in big cats of the AN/EU group except for those three belonging to the “<2” age category (*n*°8,9,10) (15/18) (Picture 11). Since the study objects of the SK group underwent a specific treatment for preservation, assessing plaque was not possible. Also, the dentition of seven big cats from the AN/EU group (*n*°4,7,11,12,16,17,18) (7/15) and two skulls from the SK group (*n*°19,21) (2/5) were covered with calculus (Picture 12). Except for three big cats of the AN/EU group (*n*°7,16,18), all specimen had some degree of gingivitis – the initial stage of periodontal disease (15/18) (Picture 13). This could obviously not be assessed among the skulls of the SK group. No study object showed clinical signs of a later stage of periodontal disease - such as gingival recession and periodontal pocket formation. Only one study object belonging to the SK group (*n*°22) was encountered with severe bone loss, presumably resulting from a periapical abscess (Picture 14-17). Last but not least, no less than six big cats of the AN/EU group (*n*°1,2,3,4,5,6) were affected with feline papilloma lesions (6/18) (picture 18). Obviously, feline papilloma lesions could not be assessed in the SK group.



Picture 10: Tooth discolouration (*n*°20)



Picture 11: Plaque (*n*°12)



Picture 12: Calculus (*n*°18)



Picture 13: Gingivitis (*n*°2)



Picture 14-17: Bone loss, probably due to periapical abscess (n°22).



Picture 18: Feline papilloma lesions on the ventral surface of the tongue (n°2).

Table 5: Acquired non-traumatic abnormalities

	Tooth Discolouration	Tooth resorption	Plaque	Calculus	Gingivitis (initial stage of PD)	GR and PPF (later stage of PD)	Bone loss (Periapical abscess)	Feline papilloma lesions
n°1			x		x			x
n°2			x		x			x
n°3			x		x			x
n°4			x	x	x			x
n°5			x		x			x
n°6			x		x			x
n°7			x	x				
n°8			x		x			
n°9			x		x			
n°10			x		x			
n°11			x	x	x			
n°12			x	x	x			
n°13					x			
n°14					x			
n°15					x			
n°16			x	x				
n°17			x	x	x			
n°18			x	x				
n°19			-	x	-			-
n°20	x		-		-			-
n°21			-	x	-			-
n°22			-		-		x	-
n°23			-		-			-
Total	1 (/23)	0 (/23)	15 (/23)	9 (/23)	15 (/23)	0 (/23)	1 (/23)	6 (/23)
AN/EU	0 (/18)	0 (/18)	0 (/18)	7 (/18)	15 (/18)	0 (/18)	0 (/18)	6 (/18)
SK	1 (/5)	0 (/5)	-	2 (/5)	-	0 (/5)	1 (/5)	-

PD: periodontal disease; GR: gingival recession; PPF: periodontal pocket formation

5 Discussion

The main goal of this study was to map the general oral health of captive Felidae and to quantitatively describe the encountered oral pathological conditions - assessed in the study objects - and identify a possible correlation between oral pathological conditions and genetics, species, age, sex, diet, behavioral and environmental aspects as well as by concomitant systemic diseases to the extent possible. Although the effect of parameters of interest in this study cannot be statistically evaluated due to the limited number of specimens included in this study, some interesting cases were encountered. Also, there exists a large variety between these specimens concerning the different parameters of interest. Relevant findings will be elaborated. Importance, critical comments and hypotheses concerning these results will be discussed and compared to the existing literature.

5.1 Traumatic abnormalities

5.1.1 Tooth fractures

The only tooth fractures encountered in this study were complicated crown fractures - fractures at the level of the crown - through enamel and dentin with pulp cavity exposure as a result (Steenkamp et al., 2018). In this study, four specimens had one such fracture of their lower or upper canine tooth. A recent prospective study in 256 captive cheetahs claimed that this type of fracture commonly occurs in big cats. Almost 75 percent of all study objects had at least one fractured canine tooth. The prevalence was positively correlated to age (Steenkamp et al., 2018). The amount of specimen in this study was too small to draw strong conclusions about this matter. However, we can mention that all four specimens with complicated crown fractures were lions, with a median age of 10.5 years. It is known that exposure of the pulp cavity increases the risk of pulpitis (Wiggs and Lobprise, 1997; Steenkamp et al., 2018). In one case, the pulp cavity was still bleeding. This suggested a recent trauma (Cvek et al., 1982; Cvek and Lundberg, 1983). Pulpitis can become clinically relevant; it may lead to pulp necrosis and may result in periapical abscesses, draining fistula's, periapical granuloma's, radicular cysts or even osteomyelitis (Olfert, 1974; Steenkamp et al., 2018).

Of all teeth, canines are most likely to undergo a complicated crown fracture, followed by premolars, carnassial teeth, and incisors. Their shape and function cause the relative fragility of canines compared to other teeth (Van Valkenburgh, 1988). Eating bones increases the risk of tooth fracture because of the high and unpredictable stress with different potency in different directions. The fact that canine teeth did not undergo evolution towards functionally and structurally more stress-resistant teeth is probably due to the importance of maintaining the current functional shape and the material properties of tooth tissues (Valkenburgh, 1988). Besides this natural impact, artificial impact may also occur in captivity. Biting on bars of their enclosure is another possible explanation for canine tooth fracture in captive Felidae (West et al., 2014).

5.1.2 Tooth attrition or abrasion

In the current study, wear was detected in eight specimens. Attrition - wear due to contact between the surface of maxillary and mandibular teeth during occlusion - was present on the carnassial teeth of one lion (*n*°21). Carnassial teeth are usually affected with this type of wear according to Steenkamp et al. (2018). The other seven specimens were diagnosed with abrasion - wear caused by contact of dental hard tissue and foreign material. Pronounced abrasion can lead to exposure of the pulp cavity. This type of wear was located on the vestibular tooth surface in a panther, tiger, cheetah and 3 lions. In one sole panther skull (*n*°23), abrasion was orientated in the occlusal plan of the canines, hereby exposing the pulp cavity.

Long-term feeding from stainless steel bowls might be an explanation for this phenomenon (Steenkamp et al., 2018). Another possible reason could be teeth grinding against the bars, walls or foreign objects of or in the enclosures of the animals. In this case, psychological health of Felidae in captivity might be questioned (Tilson et al., 1994; Szokalski et al., 2012). This may be countered by providing even more species specific enrichment of the enclosures.

5.1.3 Tooth absence (avulsion)

Tooth absence can be congenital or acquired - mostly due to trauma (tooth avulsion). Steenkamp et al. (2018) most frequently detected tooth absence of the first two maxillary incisors (101, 102, 201 & 202) and the second maxillary premolars (106 and 206). Four specimen included in this study were detected with tooth absence. In both cheetahs, absence of some incisors were seen. These cheetahs were 14 and 16 years old at the time of euthanasia. No dental trauma was recorded in their medical history. Absence of incisors and canines were seen in both panthers. Unfortunately, these specimens belong to the SK group; medical history and age are unknown. Therefore, further statements about this matter could not be made. Another critical comment may be that these skulls belong to a private collection. This makes it impossible to state whether these teeth were already lost during their life, lost during the specific treatment for preservation or even lost during the actual preservation of the skull. Risk of tooth absence may obviously increase with aging. Both in this study and the African study, tooth absence was difficult to interpret since no radiographic examinations were performed. However, the study of Steenkamp et al. (2018) included follow up visits during several years after the first visit, confirming trauma to be the most common cause for tooth absence.

5.1.4 Mucosal ulcerations

Mucosal ulcerations could be found on the hard palate in eight specimens of the AN/EU group. Obviously, this was impossible to assess in the skulls. Although not proven, trauma during feeding is presumably the most logical aetiology for these lesions. This opinion is shared by Steenkamp et al. (2018). Severe lesions might cause inflammatory reactions but this won't provoke too much complications in captive Felidae. Considering they receive the same diet as wild Felidae, their body should physiologically be able to cope with this kind of natural impact.

5.2 Acquired non-traumatic abnormalities

5.2.1 Tooth discolouration

Discoloration of teeth can occur due to diverse intrinsic and extrinsic influences (Joiner et al., 2008). Local extrinsic stains on the enamel surface can be caused by different pigments. In small animal practise, these stains can be easily removed by scale and polish dental care. For obvious practical reasons this is not feasible in captive carnivorous animals such as big cats. Nevertheless, the presence of structural enamel defects, either acquired or due to developmental abnormalities, can lead to internalized discoloration (Watts and Addy, 2001). Thickness and structural properties determine the amount of light absorption in the enamel. Because of the translucent aspect of the enamel, structural properties and colour of the dentin also have a great influence on the final colour of the tooth (Yu et al., 2009). Intrinsic tooth discoloration can be the result of a variety of developmental or acquired and local or systemic causes (Boy et al., 2016). Systemic causes include tetracycline usage. This antibiotic drug irreversibly forms complexes with dentin compounds - tetracycline-calcium orthophosphate complexes - causing yellow to brown discoloration of the teeth when exposed to light due to oxidation (Shwachman and Schuster, 1956; van der Bijl and Pitigoi-Aron, 1995; Boy et al., 2016). During gestation, the calcification of deciduous teeth takes place. Calcification of permanent teeth starts after birth and is completed before adult age is reached (Sánchez et al., 2004). Tetracycline administration during gestation can lead to discoloured deciduous teeth of neonati. However, tooth exfoliation will result in normal coloured permanent teeth (Cohlan, S.Q., 1977). In this study, discoloration was only noticed in the dentition of one lion skull ($n=20$). Since the discoloration was yellow to brown, adverse drug reaction of tetracycline usage is the most likely aetiology. Intraoral radiographs of this specimen were taken in order to estimate the age at time of death. Odontoblasts, located at the periphery of the pulp, continue to produce (secondary) dentin in vital teeth. This implies that teeth of older cats have pulp cavities which are more narrow and dentinal walls which are thicker compared with permanent teeth of young cats (Morse, 1991; Orsini and Hennes, 1992; Gracis, 2007; Park et al., 2014; Reiter and Soltero-Rivera, 2014). The dental radiographs showed a permanent dentition with very narrow pulp cavity (Supplement 3). Therefore, we can state that the specimen reached an advanced age. Not only the skull, but also the entire skeleton of this specimen was available in a private collection. Tetracyclines are known to be incorporated in all tissues undergoing calcification (van der Bijl and Pitigoi-Aron, 1995). Discoloration of bone was not observed in the overall skeleton. However, that finding does not exclude the hypothesis of prior tetracycline use. Absence of discoloration of the rest of the skeleton can be explained by the high turn-over of bone tissue (Grynopas, 1993). Once primary dentin is formed, its structural properties and colour are considered permanent because there is no remodelling during life (Morse, 1991). All of this information taken into account, tetracycline administration at a young age would be a plausible explanation for the tooth discoloration in this specimen.

5.2.2 Tooth resorption

Tooth resorption is a well-described oral condition in several species of big cats. The prevalence of dental resorption in wild Felidae is reported to be lower compared to domestic cats (Kertesz, 1993; Berger et al., 1996; Roux et al., 2009; Pettersson, 2010; Niemiec and FAVD, 2012; Aghashani et al., 2016; Aghashani et al., 2017; Steenkamp et al., 2018). The exact aetiology is unknown but this

condition is characterized by enamel and dentin resorption of the affected teeth due to the activation of odontoclasts (Niemić and FAVD, 2012). Evidence has been presented suggesting tooth resorption increases with aging (Roux et al., 2009; Aghashani et al., 2017). Since most studies - reporting prevalence of tooth resorption in big cats - assess a younger population compared to studies in domestic cats, this might be a partial explanation of the higher incidence in domestic cats. The hypothesis of a specific dental ultra-structure of domestic cats which predisposes to the development of tooth resorption might be interesting to investigate in further studies (Pettersson, 2010). Statements about tooth resorption cannot be made based on this study considering no study object was clinically affected. In the study of Steenkamp et al. (2018), on the other hand, some cases (data not presented) of tooth resorption in cheetahs were encountered. Unfortunately, presence and extent of the condition in each individual case could not be evaluated because no equipment for intraoral radiography was available (Verstraete et al., 1998).

5.2.3 Periodontal health

When abundant, plaque - a biofilm or protective community of bacteria - can be seen with the naked eye as an opaque soft adherent layer on the tooth surface. Plaque bacteria provoke gingivitis. Calculus (tartar) - the result of mineralized plaque - can be observed as a rough and hard dental deposit. This rough surface promotes plaque accumulation. Therefore, calculus is seen as a predisposing factor of periodontal disease (Perry and Tutt, 2015). The study objects of the SK group underwent a specific treatment for preservation which resolved plaque but leaves calculus untouched. Except for three specimens belonging to the "<2" age category, all other specimen of the AN/EU group had obvious plaque on their dentition. Respectively one specimen of the "6-10" age category and six of the "11-20" age category, belonging to the AN/EU group, showed dentition containing calculus. Also, calculus was recorded in two skulls. Age was unknown in one of both skulls but the other belonged to the "11-20" age category. Currently only little information about these pathological conditions in captive big cats can be found in the existing literature. Relying on the results of this study, one could claim that the presence of plaque and calculus increases with age. This is not surprising considering plaque and calculus pile up when no prophylactic tooth cleaning, let alone consistent daily thorough dental care like in small animal practice, is being performed (Perry and Tutt, 2015). The physical character of the diet has a big contribution to plaque and calculus formation. In other words, soft texture diets do not provide sufficient abrasive activity on the teeth surface. Fresh meat on the bone, on the other hand, can counter plaque and calculus formation (Miles and Grigson, 2003). All big cats included in this study and enclosed in EAZA zoos received a diet which meets this requirement.

Except for the tiger and both cheetahs in this study, all other specimens of the AN/EU group showed some degree of gingivitis. Some oral physiological or pathological conditions (tooth exfoliation, plaque and calculus, tooth fractures, tooth avulsions, etc.) might be the triggering factor for this phenomenon. In order to prevent biased conclusions, we have to mention that the big cats without gingivitis were all recently euthanized before being examined. Post-mortem pale mucosae might explain why there was no degree of gingivitis seen in these animals. Gingivitis is the initial stage of periodontal disease and can only be reversed by consistent daily thorough dental care, as advised in small animal practice (Perry and Tutt, 2015). Unfortunately this is not feasible in captive carnivorous animals for obvious practical reasons.

The later stage of periodontal disease is characterized by gingival recession, periodontal pocket formation, or both. No such conditions were observed in the study objects included in this study. In contrast to tooth resorption, periodontal disease in big cats is poorly described in the existing literature. Periodontal disease is the most common oral pathological condition in domestic cats (Perry and Tutt, 2015). Wild Felidae species would be less likely to develop this disease, because they strictly feed themselves with fresh meat on the bone (Miles and Grigson, 2003). All captive big cats of the AN/EU group in this study were also fed a strict carnivorous diet. Therefore, this study can support the hypothesis of the periodontal disease - free state of the majority of captive Felidae (Miles and Grigson, 2003). Incidence of this disease is claimed to increase with aging according to Steenkamp et al. (2018).

Prophylactic tooth cleaning in these captive big cats when under anesthesia might counter plaque and calculus formation and contribute to better gingival and periodontal health (Haberstroh et al., 1984; Miles and Grigson, 2003). To date, this is not a standard protocol in the EAZA zoos participating in this study.

5.2.4 Periapical abscess

One panther skull ($n=22$) showed bone loss of its facial bones departing from the alveolar bone of its right upper canine. Right incisive bone, both maxillary bones, both nasal bones and both frontal bones are involved in this pathological process. A likely explanation for this pathological phenomenon would be a complicated crown fracture of the upper right canine with pulp cavity exposure and probably pulpitis as a result. Pulpitis will have led to pulp necrosis, resulting into a periapical abscess and - given the visible bone damage on the skull - probably even osteomyelitis. No periapical abscess and complicating osteomyelitis of this proportion in a big cat is currently described in the existing literature. Therefore, we can merely document this case.

5.2.5 Feline papilloma lesions

Six specimen belonging to the AN/EU group showed exophytic proliferative papillary lesions on the ventral surface of their tongue compatible with Feline papilloma virus (FPV). FPV lesions seen in the study of Steenkamp et al. (2018), showed the same anatomical location. However, histology should be performed in these cases in order to properly diagnose these affected big cats with FPV (Sundberg et al., 2000). Immunodeficiency is claimed to be a predisposing factor in mammalian species for papillomavirus infections (Sundberg and O'Banion, 1989; Sundberg et al., 2000). All of these affected lions live in enclosures in the same zoo. No study object encountered in that zoo was lesion - free. The age of these lions ranges between 4 to 13 years old. This is not surprising considering the contagious nature of these viruses. Feline papilloma viruses are documented in different species of big cats (Sundberg et al., 2000; Rector et al., 2007).

5.3 Genetics

Calculating the coefficient of inbreeding of each individual cat using Wright's formula would be the ideal strategy to obtain a quantitative parameter of inbreeding in this study. Unfortunately this was not feasible due to a lack of available pedigree information of each individual animal. EAZA was founded in 1992 and has launched breeding programmes for different species - among which big cats. Therefore, we assume that the coefficient of inbreeding is negligible for each big cat born since 1992 - in all of the associated zoos. As for the skulls, origin and age were unknown except for the skull offered by Zoo Antwerp. Thus, conclusions about the coefficient of inbreeding could not be made.

6 Conclusion

Based on this study, the overall oral health of captive Felidae in the participating EAZA zoos is rather good. No congenital abnormalities were observed. Traumatic abnormalities - such as tooth fractures, tooth attrition or abrasion and tooth absence (presumably tooth avulsion due to trauma) - on the other hand were sporadically seen. Furthermore, some acquired non-traumatic oral pathological conditions - such as plaque, calculus, gingivitis and feline papilloma lesions - were frequently encountered.

Prophylactic tooth cleaning in captive large felids when under anesthesia might be considered by the EAZA zoos participating in this study in order to achieve better gingival and periodontal health. To date, this is not a standard protocol in the EAZA zoos participating in this study.

This pilot study may contribute to improving the knowledge of the general oral health of Felidae in captivity – and in EAZA zoos in particular. Hopefully, new information about this matter will stimulate more in-depth investigations and may ultimately lead to the improvement of animal welfare and breeding programmes.

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8 Supplements

Supplement 1: Dedicated form used in this study.



GENERAL INFORMATION		
Origin & type		Zoo: anesthesia
		Zoo: euthanasia
		Zoo: skull
		Private collection: skull
Species		Panthera leo leo
		Panthera leo persica
		Panthera tigris
		Acinonyx jubatus jubatus
		Panthera pardus
Age		Other: specify
		Age in years
Sex		Unknown
		Male
Weight		Neutered male
		Female
		Neutered female
		Unknown
Weight		Weight in kilogram
		Unknown
NUTRITION*		
Days fed/week		Number of feedingdays per week
Diet		Soft and humid: pure raw meat
		Intermediate: combination of bone with raw meat
		Hard and dry: pure bone (theoretically)
Supplements		Not given
		Given: specify

*Not assessible in skulls from private collections

ORAL PATHOLOGIES	
CONGENITAL ABNORMALITIES	
Mandibular mesiocclusion	Absent
	Present
Mandibular distocclusion	Absent
	Present
Palatoschisis	Absent
	Present
Hyperdontia	Absent
	Present
Hypodontia	Absent
	Present
Microdontia	Absent
	Present
TRAUMATIC ABNORMALITIES	
Tooth fractures	Absent
	Present: tooth number(s) (Triadan system)
Tooth attrition/abrasion	Absent
	Present: tooth number(s) (Triadan system)
Tooth avulsion	Absent
	Present: tooth number(s) (Triadan system)
Mucosal ulcerations*	Absent
	Present
ACQUIRED NON-TRAUMATIC ABNORMALITIES	
Tooth discolouration	Absent
	Present
Tooth resorption	Absent
	Present
Plaque*	Absent
	Present
Calculus	Absent
	Present
Clinical signs of the initial stage of periodontal disease: gingivitis*	Absent
	Present
Clinical signs of a later stage of periodontal disease: gingival recession, periodontal pocket formation*	Absent
	Present
Clinical signs of a periapical abscess: bone loss	Absent
	Present
Feline papilloma lesions*	Absent
	Present
<i>Other oral pathology: specify</i>	Absent
	Present

*Not assessible in skulls

MEDICAL HISTORY

PEDIGREE INFORMATION

COEFFICIENT OF INBREEDING

WRIGHT'S FORMULA:

$$F_i = \sum \left[\left(\frac{1}{2} \right)^{n_1+n_2+1} (1 + F_a) \right]$$

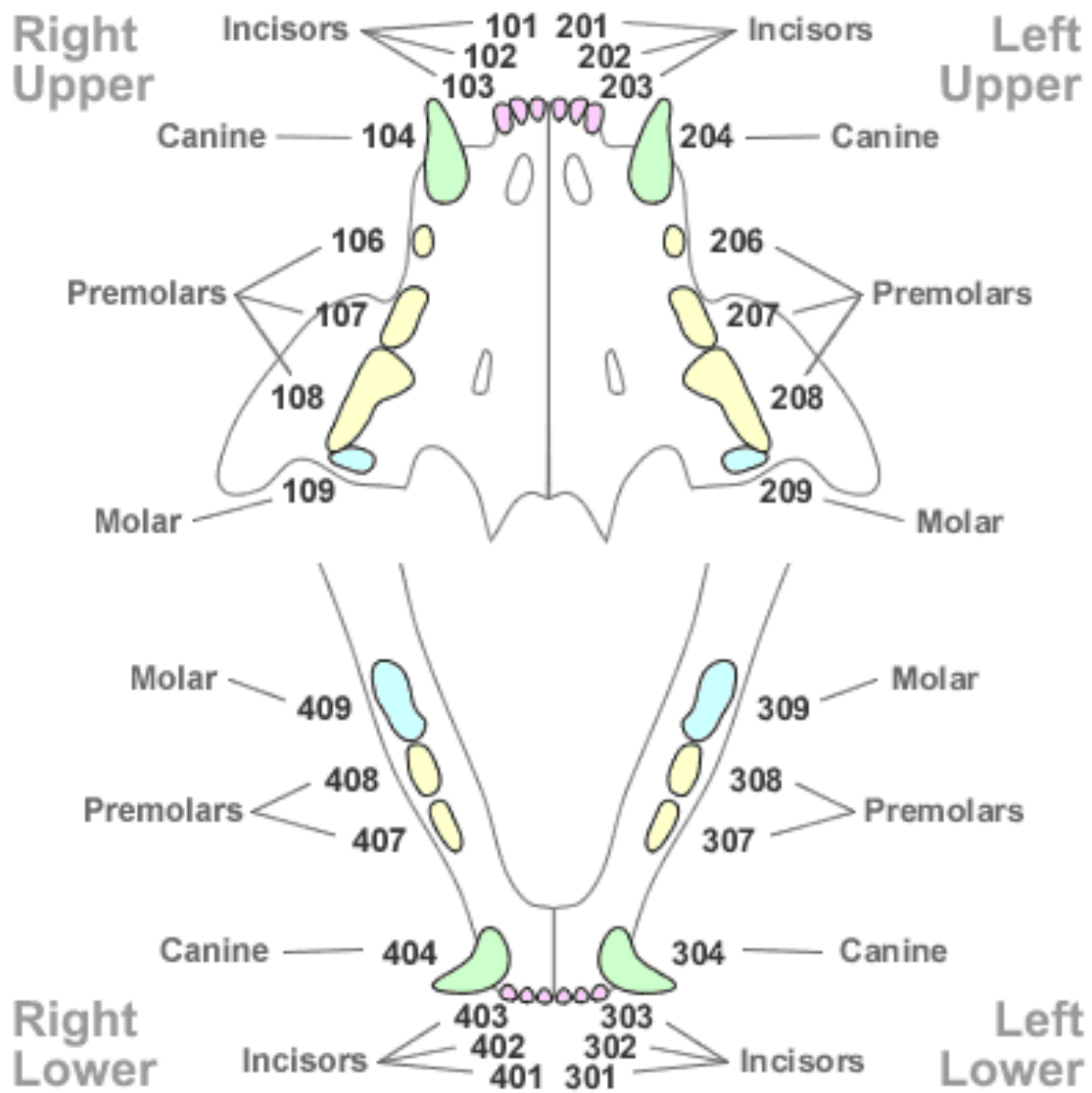
F_i : coefficient of inbreeding of individual i

F_a : coefficient of inbreeding of the common ancestor

n_1 : number of generations in the inbreeding path from the father of individual i to the common ancestor

n_2 : number of generations in the inbreeding path from the mother of individual i to the common ancestor

Supplement 2: The modified TRIADAN system for the classification of dentition in the domestic cat.



(Crossley, 2002)

Supplement 3: Dental radiographs of the lion skull (n°20) affected with tooth discolouration.

