Degenerative Joint Disease in Captive Large Cats

the radiographic characteristics and clinical presentation of DJD in captive cheetahs (*Acinonyx jubatus*), lions (*Panthera leo*) and tigers (*Panthera tigris*)

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DECLARATION

This thesis is submitted to The University of Sydney in fulfilment of the requirements for thedegree of Doctor of Philosophy. I hereby declare that I have not submitted this material, in either full or in part, for a degree at this or any other institution.

This is to certify that to the best of my knowledge, the intellectual content of this thesis is the product of my own work, and that all the assistance received in preparing this thesis and sources have been acknowledged in the text.

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ABBREVIATIONS

AF	annulus fibrosis
AS	ankylosing spondylitis
CD	chondrodystrophic
CEP	cartilaginous endplate
CT	computed tomography
DDD	degenerative disc disease
DICOM	Digital Imaging and Communications in Medicine
DISH	diffuse idiopathic skeletal hyperostosis
DJD	degenerative joint disease
DLSS	degenerative lumbosacral stenosis
EP	endplate
HD	hip dysplasia
IDD	intervertebral disc degeneration
IVDD	intervertebral disc disease
LFL	left forelimb
LHL	left hindlimb
LSD	least significant difference
MRI	magnetic resonance imaging
NCD	non chondrodystrophic
NP	nucleus pulposus
OA	osteoarthritis

OC	osteochondrosis
OCD	osteochondritis dissecans
RA	rheumatoid arthritis
RFL	right forelimb
RHL	right hindlimb
se	standard error of the mean

ABSTRACT

Lions, tigers, and cheetahs are commonly held in zoos, experiencing greater longevity in captivity compared with the wild. As a result, they are more likely to develop age-related diseases, including degenerative joint disease (DJD), during their lifetime. However, relatively little is known about DJD in these species. As DJD is an important welfare concern, zoo clinicians would benefit from knowledge regarding the radiographic prevalence and characteristics of DJD, predictors for DJD development, and the spectrum of DJD-associated signs for these species.

To obtain this information, thirteen zoos and one referral veterinary teaching hospital provided radiographic studies and matched clinical records for all cheetahs, lions and tigers that underwent imaging between 1979-2019. Radiographs were reviewed and arthropathies of the axial and appendicular skeleton were identified, with their severity scored. A generalised linear mixed model with an underlying binomial distribution ($P \leq 0.05$) examined correlation between radiographic arthropathy status and a range of potential predictors for arthropathy detection. Radiographic DJD status was then matched with clinical record entries to investigate the clinical signs of DJD in these three species, with a three-level random-effects logistic regression model used to explore the correlation between the clinical signs at presentation of all large cats that underwent skeletal imaging, against a range of predictors including radiographic DJD status.

Arthropathies were found to be common and almost exclusively degenerative in nature. Increasing age was the strongest predictor for joint disease, and a significantly lower prevalence of disease was recorded for the cheetah. Whilst all species showed unique patterns of disease, there were many similarities between the lion and tiger. In particular, severe axial DJD was a feature for both geriatric lions and tigers. There was a conspicuous absence of appendicular DJD detected in older cheetahs, however axial DJD is reported in this species for the first time, with both increased prevalence and severity of axial disease found in cheetahs from urban compared with open-range zoos. The meniscal ossicle and supinator sesamoid bone were identified as normal structures that become radiographically evident with skeletal maturation, with the supinator sesamoid bone reported in the tiger, and the meniscal ossicle in the Sumatran tiger, for the first time. A spectrum of DJD-associated presenting clinical signs, encompassing changes in gait and posture, abnormal orthopaedic evaluation and mobility impairment consistent with musculoskeletal pain, was established for the three species.

Combined these findings provide zoo veterinarians with clinically relevant information regarding the prevalence, distribution, severity and clinical presentation of radiographic DJD in captive cheetahs, lions and tigers. This research will both facilitate radiographic diagnosis of DJD and enhance understanding of the clinical impact of this disease in captive nondomestic felids, whilst also providing impetus for further research in this area.

Chapter 1

Introduction and literature review

1.1 Introduction

This literature review examines the current status of large cats in captivity and provides a summary of the available literature on arthropathies of large cats, both captive and free ranging, with the focus on age-related degenerative joint disease. Whilst literature pertaining to the lion, tiger and cheetah are emphasised, where appropriate other large cat species are referenced. Previous and current understanding of the pathogenesis of degenerative arthropathies is reviewed, and the spectrum of associated clinical signs, and utility of radiography as a diagnostic tool, is discussed.

1.2 The family Felidae and 'large cats'

The family Felidae encompasses all cats, with the most recent classification recognising a total of 14 genera, 41 species and 80 subspecies.[1] The most familiar member is the domestic cat (*Felis catus*), with all other species collectively referred to as variously nondomestic felids or wild cats. Nondomestic felids represent a diverse subset of the Family, ranging from the small European or Caucasian wildcat (*Felis silvestris*) to the largest of cats, the Siberian tiger (*Panthera tigris altaica*).[2] In literature, the term large or big cat has been loosely applied within the nondomestic felid cohort. In broadest terms, 'large cat' pertains to any nondomestic felid greater than 10kg bodyweight,[3] though more commonly it applies to the five extant members of the genus *Panthera*, namely the tiger, lion (*Panthera leo*), jaguar (*Panthera onca*), leopard (*Panthera pardus*), and snow leopard (*Panthera uncia*). However, a more expansive definition, that includes species beyond the Panthera genus, such as the cougar or puma (*Puma concolor*), clouded leopard (*Neofelis nebulosi*), Sunda clouded leopard (*Neofelis diardi*), and cheetah (*Acinonyx jubatus*), is equally applied.[4] For the purposes of this thesis, this expanded definition, inclusive of the cheetah, is utilised.

1.3 Large cats in captivity

Large cats have been kept in captivity for over 4000 years, with the earliest recorded Egyptian hieroglyphic evidence of cheetahs kept in captivity dating back to 2500 BC, the Mesopotamian records of captive lions predating 2000BC, and Han dynasty records of tigers held in menageries dating from 200BC. Exclusive, private animal collections persisted until the late 18th century, which saw the arrival of the precursor to the modern zoo with the opening of the Jardin des Plantes in Paris in 1793, closely followed by the Zoological Society of London (ZSL) London Zoo in 1828. These new institutions, whose philosophy was the assemblage of a collection of animals with each genus represented, heralded the transition from animals kept in captivity for the amusement of a select few, to institutions of scientific discovery accessible to the wider public.[5] As a result, zoos now represent one of the oldest surviving public attractions in modern day life, and the inclusion of large cats has been a constant, as befits their status as charismatic megafauna.[6]

However, the role and priority of zoos has changed over the last 200 years to a primary focus on conservation, in both an attempt to address the mounting imperative for biodiversity preservation, and in response to shifting societal expectations.[7; 8] Whilst holding as diverse a collection as possible, there is increased focus on, and resources directed to, those species whose wild populations are now considered threatened, vulnerable or endangered. The International Union for Conservation of Nature and Natural Resources (IUCN) Red List transcribed as of March 2020, lists lions and cheetahs as vulnerable, and tigers endangered. The current population trend for all three species is reported to be 'decreasing'. As a result, the cheetah, lion, and tiger are all considered to be at a high to very high risk of global extinction.[9-11]

Despite the expense, challenges and potential risk of housing wild felids, as a reflection of their current conservation status, large cats remain in high numbers amongst zoo collections globally.[6; 12] Estimates derived from the Zoological Information Management System (ZIMS) database (Species360, Bloomington, Minneapolis, USA)[13] show that lions, tigers and cheetahs are the three most commonly held Felidae species, collectively accounting for 47% of all Felidae currently

residing in zoos. Results of census data obtained from Species360(ZIMS) in April 2020, combined with IUCN Red List conservation status as of April 2021, for the three species of interest, and all other members of family Felidae, are presented in Appendix 1.1.

There is a general consensus that captive cheetahs, lions and tigers live longer than their free-ranging counterparts,[14-18] however evidence for this observation has only recently become available.[19] Survivorship statistics demonstrate that the average survival time for free-ranging animals is considerably less than that for captive, with the discrepancy most apparent for the tiger (Appendix 1.2). However, notably whilst captive cheetahs also outlive their wild conspecifics,[20] as a species they do not achieve the extended longevity in captivity as recorded for the lion and tiger.[21] One of the many implications of increased longevity associated with captivity is an increased incidence of age related disease in zoo collections. Diseases of note have been neoplastic or degenerative in nature, with degenerative joint disease identified as a key area of concern in ageing captive large cat populations.[15; 16; 18]

1.4 Terminology of degenerative arthropathies

Before presenting the literature regarding degenerative arthropathies in large cats, a discussion of the terminology applied to degenerative arthropathies is necessary. It has been noted that the terminology is frequently confusing and inconsistent, with a need for standardisation of both definitions and classification systems.[22] Nomenclature varies between human and veterinary literature, between the axial and appendicular skeleton, and definitions have altered over the course of published literature. Of particular relevance is clarification of the two most frequently applied terms to degenerative arthropathies of the skeleton, osteoarthritis (OA) and degenerative joint disease (DJD). As much research regarding these diseases is conducted in the human field with the use of animal models,[23; 24] both human and veterinary literature is addressed.

1.4.1 The appendicular skeleton

1.4.1.1 Terminology used in human medicine

Historically , degenerative arthropathies of the human appendicular skeleton have been known by many terms, including osteoarthritis, osteoarthrosis and degenerative joint disease , with the title 'osteoarthritis' first proposed in 1890.[25] Early in the 20th century, human arthritis research recognised the division of arthritides into two major categories: the erosive diseases, that had a major inflammatory component and principally affected younger age groups, and the contrasting DJD or osteoarthrosis/OA group, where bone hypertrophy and cartilage degeneration were found to affect an older cohort.[26] Despite a wealth of research in the intervening years, and major advances in erosive arthritides, the OA/DJD category has struggled to be defined.[25] A recent review presented draft definitions of OA from four leading organisations.[22] Included, and representative of the spectrum of draft definitions, was that from the Subcommittee on Osteoarthritis of the American College of Rheumatology Diagnostic and Therapeutic Criteria Committee, where OA was defined as "a heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins".[27] In addition, WHO recognised that "clinically, the condition is characterised by joint pain, tenderness, limitation of movement, crepitus, occasional effusion, and variable degrees of local inflammation".[28]

Recent research has acknowledged the role of inflammation in the pathogenesis of OA.[29] As a result, many authors now distinguish between OA and DJD, and consider DJD to be a misleading term that oversimplifies a complex multifactorial disease, and inaccurately implies that OA is the inevitable result of ageing or wear and tear.[26] Yet this view has been rejected by others who consider OA either a non-inflammatory disorder,[25] or to involve only minimal and microscopic inflammation, particularly compared with the inflammatory arthritides.[30] This alternative viewpoint concluded that the term OA, although convenient and having greater popular appeal, is a less accurate term than DJD. Consequently, in human literature, these two differing terminologies are both applied to the common pathology seen in the range of disorders that comprise the degenerative arthritides, and although the term OA is more widely adopted, it is not unusual for the two terms to be used interchangeably.[30; 31]

1.4.1.2 Terminology used in companion animal medicine

Terminology of degenerative appendicular arthropathies in the veterinary literature is equally confusing and inconsistent. A review of degenerative arthropathies of the feline skeleton noted that both OA and DJD were reported as study outcomes, with definitions either not provided, or when stated, differing between studies.[32] However, there is almost universal agreement that OA and DJD are common arthropathies of domestic cats and dogs,[33] can be caused by a combination of factors, and are primarily degenerative.

Different applications of the terms OA and DJD are noted. The terms may be used synonymously, with the resultant hybrid term OA/DJD defined as "the aberrant repair and eventual degradation of articular cartilage in association with alterations in subchondral bone metabolism, periarticular osteophytosis, and a variable degree of synovial inflammation".[34] Alternatively, either DJD or OA is used exclusively,[33; 35-38] and whilst the level of nomenclature clarification varies, similarities between the definitions provided for OA and DJD are apparent.[32; 36] In contrast, in some domestic cat studies, a clear distinction between OA and DJD is made. The differentiation exists when degenerative pathology is confined to peri-or juxta-articular soft tissue structures, in the absence of the intraarticular changes associated with OA. In this scenario, the criteria for DJD, but not OA, are met, with the inference that all OA constitutes DJD, but the reverse does not apply.[39; 40]

1.4.1.3 Terminology used in nondomestic felid medicine

The literature detailing degenerative arthropathies in the appendicular skeleton of large cats is sparse, and confined to a small number of surveys, and isolated case studies. That said, as with the literature for domestic cats and dogs, the terms OA and DJD have both been applied to these animals, either interchangeably,[15; 41; 42] or confined to either OA,[20; 43-46] or DJD.[47-52]

1.4.2 The axial skeleton: Human versus veterinary terminology

As with the synovial joint of the appendicular skeleton, conflicting terminologies are applied to the differing pathologies of the intervertebral joint of the axial skeleton. As the intervertebral disc is most commonly implicated, much of the terminology applied to degenerative arthropathies of the axial skeleton relate to this structure. Whilst both human and veterinary literature refer to intervertebral disc degeneration (IDD),[53; 54] intervertebral disc disease (IVDD) is infrequently used in human literature, yet is commonplace in veterinary texts, despite being considered by some to be nonspecific, ill-defined and used indiscriminately. Reporting of disc displacement (herniation, protrusion, extrusion, bulge) is also considered inconsistent and at times inaccurate. A revised and standardised nomenclature for intervertebral disc pathology in both human and veterinary literature, although proposed, has yet to be adopted.[55] Particularly dated is the classification of disc degeneration. Whilst pioneering research on canine disc pathology from the mid-20th century[56] is still commonly applied to dogs and other species, this categorisation is now questioned,[57] however there is a lack of consensus regarding appropriate alternative definitions and descriptors.

In veterinary literature, the term OA is confined to disease of the facet joint, and the more global terms DJD, or spinal degenerative disease, are frequently used to describe degenerative arthropathies of the spine. However, differing criteria for spinal DJD are reported,[32; 39] and the term is not universally applied, being conspicuously absent from several comprehensive musculoskeletal texts.[58; 59] In comparison, human literature recognises a wider spectrum of degenerative bony spinal diseases,[60] and pathologies tend to be assigned to specific disease categories. The term 'DJD' is rarely applied to the spine, but in contrast, there has been a recent proposal that, due to structural and molecular similarities between articular cartilage and the intervertebral disc, OA be adopted as the appropriate umbrella term to apply to degenerative arthropathies of the intervertebral joint(s).[61]

Of particular confusion is application of the term spondyloarthropathy. The spondyloarthropathies, extensively reviewed in human literature, are a category of inflammatory rheumatic diseases involving the axial and/or appendicular joints, with, critically, potential

involvement of additional body systems.[62] Thus, there are clear guidelines for the application of this term in human medical literature. However, spondyloarthropathy has also been applied to degenerative spinal changes in large and domestic cats, without histopathological demonstration of a corresponding inflammatory component.[63; 64] Examined with the benefit of current knowledge, this terminology is now questioned, and 'spondylosis deformans' and 'facet DJD' have been suggested as more appropriate descriptors.[32] Equally spondylosis deformans is now considered the appropriate term to describe the common finding of new bone on the lateral and ventral aspects of vertebrae,[39; 65] compared with the previously reported 'spondyloarthropathy'[64] or 'vertebral osteophytosis'.[66] This is consistent with the current view that, although not infrequently recognised in human medicine, true spondyloarthropathies are considered rare in domestic animals,[39] and this term should therefore be reserved for cases where the association between microscopic and radiographic changes, and concurrent disease, have been evaluated.

As with the appendicular skeleton, there is a paucity of literature examining degenerative arthropathies of the axial skeleton of large cats. However, with the exception of the term spondyloarthropathy, the most common umbrella terms applied are degenerative spondylosis, synonymous with spondylosis deformans and ankylosing spondylosis [67] or degenerative spinal disease, to describe any combination of findings consistent with IVDD and/or spondylosis.[41; 68-70]

In conclusion, dependent on the publication era, intended target audience or author(s), the terms OA and DJD have been used both synonymously, and to infer very different pathologies. This inconsistency hinders both interpretation of findings and comparison between studies. Whilst it is generally considered that OA is restricted to degenerative intraarticular pathology of the synovial joints of the appendicular skeleton and the facet joint of the axial skeleton, DJD can apply to both non-osteoarthritic joint-associated degeneration and to degeneration of the fibrocartilaginous intervertebral joints of the axial skeleton.[32; 40; 71] It is for these reasons that studies covering both the appendicular and axial skeleton are more likely to report DJD as the outcome of interest.[37; 39; 64; 72-74] Consequently, due to the scope of research conducted for this dissertation, the term DJD has been employed to encompass degenerative arthropathies of both the appendicular and axial skeleton. However, in the following sections of this literature review,

when discussing or describing published findings, the terminology used will adhere to either that of the cited authors, or when inconsistency precludes, the hybrid term OA/DJD will be used.

1.5 Evidence for degenerative arthropathies in large cats

1.5.1 Degenerative arthropathies of captive large cats

Many wild mammals held in captivity live longer than their free-ranging counterparts.[16] As a result of this increased longevity, degenerative diseases are now thought to be relatively common in a spectrum of animals, with degenerative arthropathies one of the four commonly described changes attributed to ageing in captive species.[5; 20; 75] Geriatric felids, particularly the larger species, are considered to be commonly impacted by arthropathies such as DJD and spondylosis [41] with subsequent and substantial impacts on health, welfare and quality of life.[18] However, despite these concerns, DJD has proved difficult to document in these animals. Zoo animal research poses many challenges, particularly for age-related disease, where systematic surveys are rare and the majority of information is gleaned opportunistically from non-standardised necropsies, resulting in small sample sizes and many confounding variables.[5; 76] As a result, there have been relatively few dedicated studies that have comprehensively documented degenerative arthropathies in captive large cats.

Osteopathological surveys of museum specimens have provided one avenue for investigation. Direct visualisation of skeletal changes has allowed assessment of both axial and appendicular arthropathies of potentially large numbers of specimens. A study of 386 skeletons representing both free-ranging and captive felids from eight species (including lion (*Panthera leo*), tiger (*Panthera tigris*) and cheetah (*Acinonyx jubatus*)) found both OA and spondyloarthropathy in captive-held large cats.[63] Equally, osteophytosis of both the spine and appendicular joints has been reported in the skeletons of seven large cat species,[14] and a comparative study of skeletons of a range of zoo mammals found that 50% of tigers and 80 % of lions were affected with similar patterns of osteophytosis.[16]

The remaining studies of captive felids are zoological institution-based, but despite increasing numbers of studies published, surveys targeting joint disease are rare. A review of the literature located only one such study, a retrospective study based on medical records, radiography and

necropsy reports, of five species of large felids (lion, tiger, leopard, snow leopard, jaguar) from a single zoological institution. Results showed that eight of the 37 included felids demonstrated degenerative spinal changes consistent IVDD or spondylosis.[68] However, as examination was confined to the axial skeleton, no information regarding pathology of the appendicular joints was available.

Morbidity and mortality studies potentially provide an alternative source of information regarding degenerative arthropathies of large felids. Although important for highlighting trends, their veterinary record basis confers some limitations, including possible bias inherent in qualitative data, and inconsistencies in practices and record keeping across multi-institutional studies.[75; 77] In particular, the reporting of arthropathies can be ambiguous, with these diseases often combined under a more generic 'musculoskeletal' categoriation. Consequently, it can be difficult to determine meaningful data pertaining to joint disease.[78] That said, arthropathies have been reported, with varying prevalence, in mortality and morbidity surveys that include captive large felids. A study examining quality of life in geriatric zoo mammals found four of 18 (22%) large felids to be affected with degenerative joint disease.[18] Similarly, in a necropsy-based morbidity and mortality study, DJD of the appendicular skeleton was reported in six of 38 (16%) captive nondomestic felids, with positive findings confined to the largest of the included species, the lion and tiger.[51]

Although a literature search failed to locate any specific morbidity studies of either captive tigers or lions, several species-specific studies of other captive felids have included arthropathies. A morbidity and mortality study of the Amur leopard detected OA in the appendicular skeleton of seven of 175 (4%) leopards, with prevalence increasing with age and the stifle and coxofemoral joints most frequently affected.[79] A retrospective morbidity and mortality study of captive jaguars in North America found that geriatric animals were affected with both appendicular joint disease and degenerative spinal disease (21% and 10% respectively), and that arthritis and IVDD were contributing factors in euthanasia for many male jaguars.[77] Finally, a recently conducted mortality study for the Eurasian lynx (*Lynx lynx*) found that DJD resulted in euthanasia for 12.5 and 11% of adults and geriatric animals respectively.[52]

In contrast, several morbidity and mortality studies have failed to identify degenerative arthropathies as a significant finding. This is particularly the case for mortality studies. In two mortality studies of lions, arthropathies were not one of the common pathologies reported at necropsy,[80; 81] and in a mortality study based on carnivore necropsy records from a single zoo (including 130 Felidae), degenerative arthropathies were not listed as a cause of mortality for any individual animal.[82] Only rarely do non-musculoskeletal health and disease studies report arthropathies as a comorbidity. However, a study of thyroid neoplasia in 10 nondomestic felids (mean age 15.9 years) found that three animals, one cougar and two leopards, were euthanased as a result of degenerative arthropathies.[83]

Finally, for a species that has been relatively intensively studied, no arthropathies have been reported for captive cheetahs across a range of morbidity, mortality and health and disease studies.[20; 84-87] Whilst it has been noted that captive cheetah populations have recorded a shortened life span in comparison to other captive large felids,[21] no association has yet been proposed between this decreased longevity in captivity and the detection and prevalence of degenerative arthropathies.

A final category for disease reporting is published case studies. However, case reports focused on degenerative arthropathies of captive large felids are sparse, and arthropathies are only infrequently identified as incidental findings in other studies. That said, tigers were overrepresented in published case reports, featuring in six of eleven reports detailing degenerative arthropathies in felids. Pathology encompassed OA of the elbow, stifle and coxofemoral joints [43; 44; 49] and degenerative spinal changes including degenerative disc disease and spondylosis, with disc displacement and its potential consequences reported.[69; 70; 88-90] In contrast, case studies of degenerative arthropathies affecting lions were confined to a single case of metabolic bone disease with secondary coxofemoral OA[50] and a paper describing fibrocartilaginous embolic myelopathy in an African lion, where demonstrable disc degeneration was the hypothesised aetiology.[91] Case reports of degenerative arthropathies in the cheetah were equally sparse, with only a single report of severe OA secondary to bilateral patella luxation identified.[45] However, degenerative arthropathies were reported in other nondomestic felids.[42; 92] Snow leopards in particular were featured, with several papers detailing the association

between OA and developmental arthropathies such as hip dysplasia (HD) and osteochondritis dissecans (OCD) of the stifle.[46; 48; 93; 94] Other nondegenerative arthropathies featured were a suspected traumatic patella luxation in a Eurasian lynx [95] and a case of acute noncompressive nucleus pulposus extrusion (ANNPE) in a Siberian tiger.[96]

1.5.2 Degenerative arthropathies of free-ranging large cats

Little is known of musculoskeletal conditions affecting free-ranging felids, particularly degenerative arthropathies.[14; 15] Suggestions for the sporadic and sparse nature of records regarding age-related diseases in wild animals centre on the shortened lifespan of free-ranging animals relative to their captive counterparts, with either mortality likely to occur before disease develops, or survival so compromised as a result, that wild animals with arthritis would not survive.[5; 15; 76] In addition, although morbidity surveys of living wild large cats have been conducted, assessment for joint disease is typically not included, and targeted arthropathy studies are notably absent from the limited number of reported health and disease surveys of free-ranging large felids.[97-100] A review of the literature also failed to locate any case studies describing degenerative arthropathies of wild large cats, with reports of joint disease confined to a single case study of a free-ranging tiger with septic monoarthropathy secondary to a penetrating wound.[101]

Conversely, and as with captive large cats, invaluable information regarding arthropathy status for free-ranging large cats has been derived from osteopathological surveys of archived specimens.[14; 63; 102; 103] Collectively, these studies have provided revealing, if contrasting results. A large study of eight species of nondomestic felids, comprising both captive and wild specimens,[63] found comparable evidence for spondyloarthropathy in the spines of both the free-ranging and captive-held cohorts. In comparison, bony changes that were classified as consistent with OA, whilst detected in captive specimens, were reported as conspicuously absent in the appendicular skeletons of free-ranging large cats. The conclusion, that wild large cats are unaffected by OA, is inconsistent with other osteopathological studies of free-ranging nondomestic felids. One of the earliest and largest osteopathological studies to include members of the Felidae family [102] reported bony changes of 'chronic arthritis' in free-ranging jaguar,

leopard, tigers and lions. Whilst the number of individual specimens affected was low, pathology could be extensive and severe, and all specimens recorded both axial and appendicular involvement. Further evidence for OA in free-ranging nondomestic felids is provided by an osteopathological study of two populations of North American pumas.[103] Not only was OA found in the appendicular skeleton of both populations, but the incidence of arthritis was found to increase with age. However, OA was not confined to aged individuals, suggesting that age was not the only causal factor for OA in free-ranging animals of this species.

Finally, whilst mortality surveys from the field have provided opportunities for joint assessment, as with health and disease surveys, only rarely are arthropathies either investigated or reported. This finding is considered partly the result of the relatively young age of the animals and their comparatively good body condition when presented for necropsy, in turn a reflection of the high incidence of premature death due to human causes.[104-106] Additionally, data from field necropsies can be compromised. Often undertaken by a range of personnel, including hunters and local biologists, as well as veterinarians, the lack of standardised necropsy protocol compounds the additional challenges of processing a carcass that has often undergone advanced autolysis prior to discovery.[20; 106] Nonetheless, occasional and opportunistic detection of degenerative arthropathies has been reported. A necropsy survey of 49 free-ranging Namibian cheetahs recorded bilateral shoulder OA in a young cheetah [20] and more compelling evidence for the existence of degenerative arthropathies in free-ranging large cats was provided by a radiological survey of the appendicular skeleton of euthanased free-ranging lions. The study detected degenerative, traumatic and septic arthropathies in nine of 15 animals. Six lions demonstrated multifocal pathology, with osteophytic reactions identified in the elbow, shoulders, and carpi, and multiple degenerative lesions in the stifle joints.[47] Consequently, this study not only presents the most substantive evidence to date that free-ranging lions develop degenerative arthropathies of their appendicular joints. These findings serve to highlight both how little is known of the prevalence of degenerative arthropathies in free-ranging large cats, and the importance of ongoing research in this area.

1.6 Causal factors for DJD in captive large cats

To date there have been no systematic studies investigating the risk factors for degenerative arthropathies in captive large cats. That said, a spectrum of studies have identified potential causal factors. Whilst these studies individually may be limited by low numbers of animals, or restricted to only a select few nondomestic felid species, as a result of the collective data generated, risk factors have been postulated, with some common themes emerging.

1.6.1 Size does matter?

Several key osteopathological studies, examining a range of nondomestic felid skeletal specimens, have reported that osteopathology was confined to the larger heavier members, the lion, tiger, leopard, jaguar, and cheetah, with smaller species such as the lynx, snow leopard and ocelot free of pathology.[102; 107] Support for these findings is provided by the conclusions from an osteopathological study of a range of large zoo mammals including lions and tigers, that morphology was a significant factor in the incidence, distribution and characteristics of degenerative arthropathies in captive zoo mammals.[16] Supporting evidence for these osteopathological findings is provided by two morbidity studies. The first, an investigation of degenerative spinal disease in five felid species – the lion, tiger, leopard, snow leopard and jaguar,[68] used clinical records, including necropsy and radiology reports, to determine that pathology was confined to the three larger species, the lion, tiger and leopard. In a second, necropsy-based, metastudy of 38 captive felids representing five species – the cheetah, lion, tiger leopard and cougar – once again, degenerative arthropathies were confined to the largest species, here the lion and tiger.[51]

It is currently unclear why larger felids, compared with the smaller species, would be more predisposed to degenerative arthropathies. However, it has been suggested that the impact of large body size can in part be attributed to corresponding anatomical features and biomechanical function, with the prevalence and distribution of arthritis in large cats associated with their body size, weight distribution and kinematics. In particular, the combination of 'jolt-shock', weight transference and locomotive power has been proposed to explain the localisation of arthritic lesions to the forelimbs in felids.[102]

1.6.2 The evidence for increasing age as a risk factor

Although degenerative joint disease is regarded as an age-related disease in large felids,[15] substantiating evidence is limited, and restricted to the findings from a small number of osteopathological and morbidity/mortality studies. Combined, these studies have reported that more extensive lesions were found in older large cat skeletal specimens,[14; 16; 102] and that the prevalence of 'arthritis', and 'musculoskeletal diseases such as spondylosis, IVDD and arthritis' increased with age, in both captive leopards [79] and jaguars [77] respectively.

1.6.3 Environmental factors

Environmental factors that may contribute to degenerative arthropathies in captive large felids are multifactorial and interrelated. Factors include the physical environment, which in turn encompasses enclosure size and design, choice of ground surface substrate and the physical structures contained within the enclosure, and management strategies, including nutritional requirements.[91] Despite this array of potential contributors, there has been little investigation into the possible impacts of any of these environmental factors.

Although enclosure design has evolved in recent times, unnatural and hard surfaces are still used. These surfaces are unforgiving compared to wild and natural substrates and have been postulated to result in abnormal force transfer to joints culminating in degenerative arthropathies.[12; 68] Despite this concern, any negative effect has yet to be proven. Equally, investigations into nutritional contributions to arthropathies in large felids are limited, with reporting confined to an isolated case study of nutritional imbalance in a lion, resulting in metabolic bone disease with concomitant unilateral coxofemoral DJD.[50] The arthropathy in this case was considered incidental. In addition, the detection of degenerative arthropathies in a spectrum of animals with a range of diets has led some researchers to conclude that diet is an unlikely causative agent for arthritides.[102; 107] In contrast, others are not as dismissive and have highlighted this as an area for further investigation.[16] Consequently, debate remains as to the potential role of nutritional imbalances in degenerative arthropathies of large cats.

A topic that has received some attention is the association between activity levels of captive large cats and the development of degenerative arthropathies. It has been postulated that the

traditional zoo environment leads to decreased levels of activity [15; 108] and equally that reduced exercise leads to a lower proportion of muscle mass, reduced muscle tone, increased subcutaneous fat and associated skeletal degradation.[17] One of the few studies of captive large cats targeting degenerative arthropathies examined the impacts of the provision of vertical climbing and feeding poles for captive tigers. This age-matched cohort study found that the incidence of degenerative arthropathies were four-fold less in tigers given access to poles, with several theories proposed for these results, including both a direct effect of improved joint health, and indirect benefits from improved muscle tone and body conditioning as a result of increased activity.[109]

1.6.4 Local factors

Case studies of degenerative arthropathies in captive large felids offer an opportunity for more detailed examination of the impact of local factors, occasionally identifying inciting causes. Most simply, local factors have been categorised as either normal forces acting on an abnormal joint such as congenital or developmental arthropathy, or abnormal forces acting on a normal joint. Of this second category, trauma would be the prime example.[71] Either scenario can lead to joint degeneration. There are several case studies that illustrate the impact of local factors on joint degeneration in captive large cats. Severe bilateral stifle OA secondary to bilateral patella luxation has been reported in a cheetah,[45] and several studies have identified OCD as a cause of stifle OA in snow leopards.[46; 48] Although the cause of the OCD lesions was unable to be determined, OCD is considered multifactorial, with traumatic, nutritional and genetic factors proposed in other species.[89; 110] Snow leopards have also been reported to suffer from another potentially inherited arthropathy, HD, which has resulted in coxofemoral DJD.[93; 94] In addition, a possible congenital cervical abnormality was advanced as a potential cause of a reported intervertebral disc extrusion in a captive held tiger.[89] However, despite trauma being occasionally suspected,[44] case studies detailing abnormal force disrupting a normal joint to the point of degeneration are notably absent from the literature.

1.6.5 Aetiology is often undetermined

The aetiology of DJD in companion animals often remains unclear.[32] This was also the case for many published reports of DJD-affected large cats. No inciting causes have either been reported or confirmed for coxofemoral, stifle or elbow OA in tigers,[43; 44; 49] and despite precedence in domestic cat literature, no causal association was proposed between severe OA and synovial osteochondromatosis in the elbow joint of a tiger.[89] In the axial skeleton, although IVDD and its manifestations have been the focus of several case reports in lions and tigers,[69; 70; 88; 91; 111] no causes for the reported intervertebral disc degeneration were reported, with the exception of an isolated case of cervical disc disease presumed secondary to congenital malformation.[89] Although heavy body weight and extended time in captivity have been proposed as possible contributing factors to disc degeneration in captive tigers,[91] there is no real evidence to support this statement.

In summary, degenerative arthropathies in captive large cats should be considered multifactorial, with the interaction between systemic and local, host and environmental factors playing a crucial role. It is also likely that aetiology may vary between the axial and appendicular skeleton and within the appendicular skeleton that local factors may play an increased role. This complex causation most likely contributes to the lack of confirmed causes in the literature.[35; 36; 112]

1.7 Pathogenesis of DJD

The majority of research investigating the pathogenesis of both OA and intervertebral joint disease has been conducted in the human field, but often utilising animal models, including species as diverse as the murine models, the domestic feline, canine, ovine and porcine models.[113; 114] Whilst not all research findings from animal models are translational to human clinical medicine, there is a significant element of interspecies commonality regarding pathogenesis. As such, the following review includes findings from both the human and veterinary fields. Additionally, although there are similarities in both molecular structure and pathological processes between the synovial and intervertebral joint, and it has been suggested that degeneration affecting these two joints be considered the same process,[61] this approach has

yet to be widely adopted. Therefore, the pathogenesis of OA, and degeneration of the intervertebral joint, will be discussed separately.

1.7.1 Appendicular skeleton: the synovial joint

The principal degenerative process affecting the synovial joints of the appendicular skeleton is OA. The facet joints of the axial skeleton, also by definition synovial joints, are equally susceptible to OA, and therefore all discussion of osteoarthritic changes and processes will similarly apply to these joints.

1.7.1.1 Pathogenesis of OA

OA is a complex and multifactorial disease,[115-117] with the currently accepted position that, although not always identifiable, all OA is secondary to a predisposing cause, capable of being triggered by a variety of inciting events or agents and should be considered the disease process that represents the final common pathway for a failing joint.[22; 34] OA is no longer considered a process of wear and tear,[118] but a pathophysiology initiated by both micro- and macro-injury,[22; 119] and although predominantly a degenerative process,[36; 39] the significance of an associated low-grade, nonpurulent inflammation is also now widely accepted.[29; 120]

OA is clinically highly heterogenous.[22; 121] Not only can different appendicular joint sites be subject to unique causative factors and aetiology, but evidence suggests that biochemical constituents of cartilage vary between joint sites [122] and that capacity for repair may differ between joints.[22] As a result, OA of different sites have been considered distinct disorders and, within the human literature, OA is often discussed at the joint level.[25; 123]

OA is now appreciated to be a whole joint disease. Although periarticular soft tissues, including ligaments, tendons, muscles and the nervous system may also be affected,[25; 26; 30; 61; 71; 119; 120] the three key structures involved in both the initiation and progression of disease are the articular cartilage, subchondral bone and synovium.[34; 124] Articular cartilage is considered the main target of pathological processes,[61; 119] with pathology characterised by progressive loss of structure and functionality. Early stages are characterised by an initial thickening of articular cartilage and hypertrophic repair. Cartilage appears oedematous as a result of increased water content due to

a damaged collagen network, with cell and tissue swelling, and proliferating chondrocytes forming clusters or clones of cells.[26; 30; 34] Although repair processes may result in new cartilage formation, integral hyaline cartilage is replaced with inferior fibrocartilage. As degradation progresses, vertical clefts or fissures develop in the articular surface, giving the characteristic appearance of fibrillation, pathognomonic for later stage OA .[30] This is accompanied by a thinning and softening of the cartilage, and a transition from the smooth, glassy, translucent white appearance of healthy cartilage to a dull ground-glass, white-grey to yellow, roughened surface.[125] Horizontal fissures at the junction of calcified and uncalcified cartilage can occur, and if combined with vertical clefts, may result in fragmentation of the cartilage surface. Fissures penetrating to the calcified zone are recognised as end-stage disease.[126] Neovascularization and innervation from the adjacent subchondral bone occur with advancing disease, with vascularization further weakening the cartilage, and innervation thought to create an additional source of pain in OA.[26; 127] Cartilage hypocellularity is a feature of advancing pathology, and ongoing loss or absence of proteoglycan in the extracellular matrix confers a loss of metachromatic staining intensity, considered by many to be pathognomonic for OA.[30; 125] Continued motion wears away the fibrillated cartilage, resulting in loss of cartilage tissue, erosion and ulceration. Ultimately there may be full thickness loss of articular cartilage, and exposure of the underlying subchondral bone,[34] inciting further pathological responses.

The importance of subchondral bone remodeling in OA is now recognised,[128] and whilst there is debate regarding changes seen in both the early and late stages of the disease,[129-131] almost exclusively [124] the hallmark feature reported for progressive OA-affected subchondral bone, is increased thickness of the subchondral plate and trabecular sclerosis.[26; 30; 34; 119; 125; 132; 133] As ulceration of articular cartilage exposes the underlying bone, subchondral bone sclerosis is often accompanied by eburnation of the bone, giving the surface the appearance of polished ivory.[26; 30] Microfractures and microcracks result in advance of the tidemark, leading to further thinning of articular cartilage and a perpetuation of the degradative process.[121] Increased penetration of vascular channels into the subchondral bone adjacent to the tidemark is seen, and intramedullary venous hypertension, as a result of microfracture remodeling, is proposed as a major cause of OA pain. Microfractures have also been associated with subchondral bone cysts, which are well

recognised structural features of subchondral bone degeneration [30; 120] and may reflect localised osteonecrosis.[26]

Osteophytes are another component of subchondral bone degeneration in later-stage OA,[134] and manifest as outgrowths or spurs of dense trabecular or compact bone, located at the margin of joints, at the interface of articular cartilage, and bone or synovium.[30; 125; 135] Periarticular bony spurs or more generalised bone proliferation cause joint enlargement, and fractured osteophytes may result in intraarticular loose bodies or joint mice. Whilst osteophyte formation varies between species,[39; 125] their production is generally considered integral to the joint remodeling seen in OA. Their formation is viewed by some to represent the joint's response to altered biomechanical stress and instability, reflecting an attempt at repair or redistribution of abnormal joint load.[30; 125] However, although the overwhelming evidence is that osteophytosis is a manifestation of OA, osteophytes in the absence of cartilage damage have been described,[135] and some authors have suggested that osteophytosis as an isolated finding may be a manifestation of ageing rather than of OA.[136]

Synovial hyperplasia and inflammation, resulting in synovitis, is now recognised to accompany the articular cartilage degeneration and subchondral bone changes of OA.[137] The degree of synovitis is variable, dependent on a range of factors including stage of disease, inciting cause and species. Although commonly noted in early disease, inflammation is generally sparse,[26; 30] however with progression the synovium undergoes villous hyperplasia and synoviocyte hypertrophy,[121] conferring a velvet appearance to the membrane. An accumulation of mononuclear inflammatory cells, with increased vascularity of the synovial membrane, may be noted,[125; 138] and variable levels of synovial capsular fibrosis may be apparent. Fragments of articular cartilage or necrotic bone from the degenerating joint surface may be incorporated within the synovial membrane.[26] Additionally, synovial inflammation per se may also result in bone erosion, with adjacent bone initially appearing ragged and spiculated, with progression to overt erosion over time.[139] Synovitis, even if only mild, can be clinically significant. The inflamed synovium is likely a key tissue in the origin of pain in OA,[140-142] and posttraumatic OA in particular may be susceptible to synovitis-induced pain.[30]

There have been no studies investigating the pathogenesis of OA in nondomestic felids. However, pathological changes reported from both clinical cases and osteopathological surveys do mirror many of the OA-associated joint changes described for other species. In particular, osteopathological surveys have reported a preponderance for osteophytic reactions and ridging, eburnation, and erosive lesions on bony articular surfaces.[14; 16; 102] In addition, gross and histopathological examination of clinically affected joints have reported a similar spectrum of pathology, including articular cartilage fibrillation, erosions leading to exposure of the subchondral bone, eburnation and subchondral bone sclerosis and synovitis.[42; 45; 51] These transspecies similarities are not surprising and highlight the many commonalities of OA pathogenesis across species.

1.7.1.2 Biomechanical implications of OA

Degeneration of individual joint components, and dysfunction of the joint as a unit, combine to create a spectrum of biomechanical alterations in the osteoarthritic joint. The increased water content seen in early OA-affected articular cartilage results in loss of the normal viscoelastic function, compressive stiffness and tensile strength properties of this structure.[34; 143] Ongoing degradation results in thinning of the cartilage layer, narrowing of the joint space, and loss of the normal contour of articular surfaces. The net result is a decreased ability to withstand diarthrodial forces and joint instability.[30] Decreased shock absorption is a feature of OA-affected joints and is a consequence of both subchondral bone sclerosis and degenerative changes to periarticular soft tissue support structures.[26; 144] The presence of osteophytes alters joint congruity, and intraarticular loose bodies from fractured osteophytes may impede range of movement. This restricted range of movement is exacerbated by any associated joint capsule thickening or fibrosis.[26] Finally, synovial joint lubricants are less effective, promoting further mechanical joint injury.[145; 146]

1.7.1.3 Radiographic changes associated with OA

Whilst there have been no systematic studies examining the radiographic features of OA in large cats, this area has been well researched in both human and companion animal medicine.[30; 34; 139] Consequently, the following discussion draws on this literature, with the addition of information relating to large cats included where available.

The trademark pathology of OA, the degradation and loss of articular cartilage, unless involving mineralisation, cannot be visualised on plain radiography.[38; 147] Whilst joint space narrowing is an indirect maker of articular cartilage loss,[148] demonstration of this feature requires weight-bearing radiography, a practice rarely applicable in veterinary, particularly exotic animal medicine.[139] As a result, conventional radiographic evaluation of OA is dependent upon the assessment of other degenerative joint changes.

Subchondral bone remodeling can be associated with a spectrum of radiographic changes. As a result of thickening and increased density of subchondral bone, increased radiographic bone opacity, recognised as a sclerotic zone several millimeters wide, may be detected.[119; 139] Whilst this sclerosis may be the first radiographic finding of OA,[26] it is frequently considered to represent more severe joint degeneration and advanced disease.[64; 119; 125; 148] Alternatively, discrete radiolucent subchondral bone defects may be detected. Representing subchondral bone cysts, they too are an indicator of chronic joint disease.[125; 139] These features must be differentiated from other late-stage OA causes of subchondral bone lucency, principally periarticular bone radiolucencies representing bone erosion from hyperplastic synovium.[139] Spontaneous diffusion of gas into the joint, referred to as the vacuum phenomenon, can also be seen in the osteoarthritic joint, and whilst not resulting in bone lucency, will cause radiographic intraarticular lucency.

Pathological changes of the synovium have proven difficult to detect radiographically in some species.[36; 40; 64] However, whether this also applies to large cats is currently unknown. That said, two broad categories of radiographic changes are reported; increased intraarticular opacity, and intra- and periarticular soft tissue swelling. These may be the result of either or both synovial effusion secondary to synovitis, or synovial hypertrophy and joint capsule thickening. Additionally, for some appendicular joints, changes in the radiographic appearance of other intraarticular structures may serve as indirect indicators of synovial effusion. The stifle joint is one example, where effusion causes compression of the radiographically identifiable infrapatellar fat pad, resulting in the so called ‘infrapatellar fat pad sign’.[38; 139; 149]

The formation of periarticular new bone, classified as either osteophytes or enthesophytes dependent on location and association with soft tissue attachment site, is a key radiographic change in osteoarthritic joints. Osteophytes are originally cartilaginous and therefore not visible radiographically. However, subsequent ossification allows radiographic detection, with a variable size and appearance noted. When superimposed over normal bone, their appearance may be limited to areas of bone of irregular density, or similarly subtle changes of irregularity or roughening of the bone margin. However, bony spurs, lipping at the edges of joints, or at their most extreme, large bony masses or exostoses protruding from the normal bone outline, are also reported.[30; 125; 139] Radiographic osteophytosis has been reported in large cats, most commonly seen in the coxofemoral and elbow joints, and affecting the lion [50], tiger [43; 44; 89] and snow leopard.[93] Osteophytosis secondary to surgical correction of an OCD lesion of the stifle in the snow leopard has also been reported.[48]

Whilst osteophytes appear radiographically similar to enthesophytes, enthesophytes are identified by their location at the entheses of intraarticular and periarticular ligaments, and the joint capsule. Thus, multiple enthesophytes may form around a synovial joint, and it can be difficult to distinguish these structures from osteophytes. However, differentiation is important, as although degenerative enthesopathies can be seen in association with osteoarthritis, their presence is not pathognomonic for the disease,[39] and in human medicine at least, enthesophytes are considered a common, nonspecific, possibly age-related finding of unclear clinical significance.[150]

Joint-associated dystrophic mineralisations can also be seen radiographically with OA, and several features fall under this umbrella term. Intraarticular osteocartilaginous bodies, postulated to occur as a result of articular surface disintegration, may either embed at a distant synovial site, or remain loose and unchanged in the joint, detectable radiographically as small, well defined and discrete articular opacities.[139] These OA-associated structures must be differentiated from other causes of intraarticular ossific bodies, such as avulsed bone fragments and small synovial osteochondromas, whose causal association with OA is still debated. Reports of intraarticular osseous bodies in large cats are limited to a single case study of bilateral elbow OA with synovial osteochondromatosis in a tiger.[89] This is distinct from the numerous studies in

nondomestic felids investigating meniscal mineralisations.[151-156] As a number of studies have confirmed their structure as bony or osseous,[151; 152; 155] they are generally referred to as meniscal ossicles. Findings concur that meniscal ossicles are frequently detected in the cranial horn of the medial meniscus of many large cat species, including the lion, Bengal tiger, and cheetah, with the classic appearance of a single, variably sized ossicle on radiography. No association has been found between the radiographic presence of meniscal ossicles and degenerative changes of the stifle joint for large cats. Consequently, in nondomestic felids they are considered a normal anatomic variant, developing progressively following birth, and detectable in skeletally mature animals. Finally, as OA is a 'whole joint' disease, involvement of other joint-associated soft tissue structures such as the joint capsule and intraarticular ligaments may occur. As a result, radiographic dystrophic soft tissue mineralisation has been detected and reported in a range of OA-affected joints.[64; 125; 157]

Whilst all of the aforementioned represent the most frequently identified radiographic changes of OA, not all are specific to OA but can also be seen with other arthropathies. For a definitive diagnosis of radiographic OA, one or more hallmark features of OA – indirect evidence of articular cartilage loss, subchondral bone remodeling, or periarticular new bone production – must be identified. In their absence, with radiographically detectable pathology confined to soft tissue, the umbrella term DJD is applied.[40]

1.7.2 Axial skeleton: the intervertebral joint

The fibrocartilaginous intervertebral joint is considered a three joint complex, consisting of the ampiarthrosic endplate-disc-endplate joint incorporating the osseous component of the endplate (EP) and referred to as the intervertebral disc complex, and the two facet joints.[61; 158; 159] The disc itself is essentially fibrocartilaginous, consisting of a central, soft and gelatinous nucleus pulposus (NP) surrounded by the fibrillar annulus fibrosus (AF), with the cartilaginous endplate (CEP), anchoring the disc to adjacent vertebral bones, generally considered to be the third component of the disc.[54; 57; 160-162]

1.7.2.1 Pathogenesis of degeneration of the intervertebral disc complex and associated structures (excluding the facet joint)

Intervertebral disc degeneration (IDD) has been described as an aberrant cell-mediated response to progressive damage, resulting in deterioration of the disc matrix and structural changes to the disc, culminating in loss of disc integrity and ultimately structural failure. Both pathological and age-related processes contribute to these changes.[53; 54; 163] The initiating events and exact mechanisms involved in intervertebral disc degeneration are not fully determined,[160; 164] however NP degeneration is a critical component. Chondrification (the replacement of NP notochordal cells with small chondrocyte-like cells) and catabolic cell behaviour result in a decrease in proteoglycan content[165] and degradation of the extracellular matrix, with the subsequent decrease in intradiscal pressure limiting the NP's ability to function as a hydraulic cushion.[166; 167] Changes to collagen type render the NP more fibrous,[166] and the characteristic gelatinous consistency of the NP can become progressively more cartilaginous and granular. The degenerated NP becomes desiccated, friable, and may calcify,[168] with the result that the now rigid disc compromises biomechanical competence, furthering mechanical damage to the NP.[167]

As a result of NP degeneration, increased stress is transferred to the AF, a structure poorly designed to resist compressive forces. Disorganisation of the annular lamellae result in increased stiffness and weakness of the AF. No longer able to contain the NP, an outward bulging of the intervertebral disc occurs when subject to physiological loading, potentially resulting in disc protrusion. A variety of clefts, tears and fissures, extending from the degenerative NP into the AF, accompany its structural failure,[158; 169] allowing extrusion of degenerated NP material. These changes further compromise function of the intervertebral disc, and a vicious cycle is perpetuated.[162]

The contribution of the CEP to disc degeneration has recently received attention. Whilst recognised as the weakest structure of the disc complex, the semi-permeable CEP plays a vital role in nutrient supply from the well-vascularised vertebral body to the relatively avascular disc. Thickening, thinning, and calcification of the CEP have all been described in the degenerative process.[54; 160; 161] Combined with sclerosis of the osseous layer of the EP, the end result is

obstruction or obliteration of vasculature beds, disrupted passive diffusion and nutrient exchange via the CEP to the disc, and progressive disc degeneration.[54; 61; 158; 161]

Focal defects have also been demonstrated in degenerating EPs,[57] with microfractures and breaches associated with intravertebral herniation of the NP (Schmorl's nodes). However, the association between Schmorl's nodes and disc degeneration remains controversial, with a recent human cadaveric study concluding that previous reports of Schmorl's nodes may actually represent differing EP pathologies, including erosions and calcifications.[170]

The association between disc degeneration and nerve ingrowth is key for recognition and understanding of discogenic pain. Whilst innervation of the healthy disc is confined to sparse nerve endings in the outer third of the AF,[171] in human 'chronic back pain' patients, disc degeneration was accompanied by both vascularization and nerve ingrowth.[172] Nerve fibres not only extended into the inner AF and NP, but also stained positively for substance P, a nociceptive neurotransmitter. As there are no equivalent studies from the veterinary literature, it is unclear whether disc degeneration in animal species results in similar pathological changes.

Whilst the sequence of degenerative changes to the disc are currently unclear, investigators agree that degenerative changes of the NP precede those of the AF.[54; 161; 173] Regardless, all degenerative processes across the disc are interactive and additive,[158] with end-stage disc degeneration characterised by a loss of differentiation between the NP and AF, creating an amorphous fibrocartilage. The NP, now devoid of its gelatinous properties, is fibrotic and opaque, the AF lamellae are disorganised and possibly ruptured, and sclerotic EPs may fracture and erode. The dehydrated disc undergoes a yellow-brown discoloration, and diminished disc height (distance between the two EPs) is evidenced as disc space narrowing.[54; 56; 158]

Disc degeneration has several relevant ramifications in veterinary medicine. The first, disc displacement, can be manifest as either disc extrusion or protrusion.[56] Secondly, disc degeneration leading to stenosis or spinal canal narrowing may result in neural compression, with degenerative lumbosacral stenosis (DLSS) in particular receiving attention.[174] A sequel to disc degeneration only recently recognised in human medicine, but infrequently addressed in the veterinary literature, is degenerative disc disease (DDD). DDD is defined as disc degeneration that

results in structural failure of the intervertebral joint and is associated with discogenic pain.[175] Representing the main cause of lower back pain in human patients, DDD is classified as a major global human health disorder.[176] The subject of some conjecture, it is currently unclear whether the condition of DDD and associated discogenic pain exist in veterinary patients.[177]

IDD alters the loading response and alignment of the vertebral column, with subsequent degeneration of facet joints, vertebral bodies, paraspinal muscles and spinal ligamentous apparatus all recognised as potential components of axial DJD.[61; 160; 164] Vertebral body changes are emphasised, and whilst human literature describes the presence of osteophytes at vertebral body margins,[160; 166] a discussion of spondylosis deformans dominates the veterinary literature. Defined as a noninflammatory degenerative condition, spondylosis deformans is characterised by the development of bony projections at the attachment site of AF Sharpey fibres to the cortical surface of adjacent vertebral bodies.[178; 179] Previously referred to as vertebral osteophytes,[66] vertebral enthesophytosis is now considered the appropriate term for spondylosis deformans.[180] Natural progression of the disease involves the expansion of enthesophytes ventrally and laterally, with resultant spurs of variable dimensions. Bony bridging of the disc space has been reported, leading to fusion of the affected vertebral column.[159] The exact pathogenesis of spondylosis deformans is unclear. Although often seen in association with disc degeneration,[159; 178; 181] no causal effect has been established, and whilst it is acknowledged that disc degeneration can lead to spondylosis deformans,[54] conversely spondylosis deformans may result in disc degeneration. In particular, a domino effect may be seen with bridging spondylosis deformans of multiple contiguous vertebrae, where increased stress associated with fusion of local spinal segments results in loss of compressibility and degeneration of adjacent discs.[182-184] This complex interplay between spondylosis deformans and disc degeneration is but one example of the interrelated nature of the various components of the spinal unit. Whilst a relatively orderly sequence of degenerative changes has been described, commencing with disc degeneration followed by secondary facet OA and spondylosis deformans,[54] it is now thought that these degenerative changes occur simultaneously, or if not, then with close temporal association.[160]

Whilst there have been no studies investigating the pathogenesis of axial DJD in captive large cats, available information, gleaned from a combination of osteopathological studies,[14; 16; 102; 109]

a single case series,[68] and a small number of published case studies,[69; 70; 88; 89; 111] does provide some insight into the spectrum of degenerative features found in these species. Although axial DJD is presumed common in large nondomestic felids,[3; 16; 41] there is little evidence to support this statement, with any reported incidence confined to observations from two small studies only.[16; 68]

However, whilst reported prevalence of disease is unclear, there is consistency across studies regarding the spectrum of reported degenerative lesions. Disc mineralisation, narrowing of the intervertebral disc space, and disc displacement, including both protrusion and extrusion, are all described.[41; 68; 185] Spondylosis is considered common in large cats,[185] however opinion is divided as to whether large cats are predisposed to bridging (ankylosing) spondylosis, with a notable absence in one study prompting speculation that the superior flexibility of the vertebral column in these species may be somewhat protective.[16] An association between spondylosis and IDD has been suggested,[68] however IDD has also been reported in isolation.[69] There are numerous reports of axial DJD leading to neurological dysfunction, with both spinal cord compression due to disc displacement,[69; 89; 185] and spinal nerve compression due to spondolytic bone expansion described.[185] Notably there are no published reports of facet OA affecting large cats.

Axial DJD in large cats is most frequently reported to be multifocal, with all levels of the vertebral column susceptible. However, clinical cases most commonly describe pathology affecting the lumbar spine and lumbosacral junction,[68-70; 88; 185] with the exception of an isolated case report of IVDD of the caudal cervical spine.[89] In comparison, osteopathological studies report a high frequency of vertebral lesions in the thoracic column.[14; 102] The discrepancy most likely reflects the limitations of the respective methodologies, with osteopathological studies confined to bony changes, and case reports generally confined to clinically significant lesions. However, the suggestion that thoracic DJD may be subclinical cannot be discounted.

1.7.2.2 Biomechanical implications of axial DJD

The intervertebral joint has unique biomechanical properties, with the facet joints integral for mechanical stabilisation of the vertebral column,[159; 186] whilst the intervertebral disc complex is the main shock absorber, distributing compressive loads between osseous segments.[166] As a

result, decreased hydrostatic pressure within the disc that accompanies NP degeneration results in one of the principal biomechanical effects of axial DJD, loss of shock absorption. Lowered intradiscal hydrostatic pressure also leads to loss of disc height. This in turn impedes the separation of, and alters the loading pattern to, contiguous vertebrae, whilst also impacting the effective operation of the associated spinal ligaments.[186]

The ensuing collapsed disc space, AF degeneration, and damage and sclerosis of the EPs all potentially contribute to the other primary biomechanical consequence of axial DJD, abnormal segmental mobility.[158] This can be manifest as both instability and, paradoxically, stiffness, with debate within the literature regarding any temporal association between the two. Whilst a two-stage process has been hypothesised, with initial instability and increased mobility of the affected spinal segment followed by stabilisation and stiffening,[54; 187] recent reviews have highlighted conflicting findings.[166; 188] That said, a trend towards spinal stiffening with increased degeneration is most commonly accepted, with collapsed intervertebral disc spaces and bridging spondylosis in particular identified as key contributors to stabilisation and stiffening of the affected axial segment.[54; 166; 188]

1.7.2.3 Radiographic changes associated with axial DJD

Whilst plain radiography has been widely recognised for its utility in the investigation of bony lesions, it is a suboptimal modality for both the evaluation of healthy discs , and with two notable exceptions, detection of degenerative changes within the disc itself.[159; 189] Firstly, radiolucent pockets within the disc, representing collections of gas and otherwise known as vacuum disc phenomena, whilst not specific to disc degeneration, can be noted.[158] However, the more commonly detected radiographic change to the disc is that of mineralisation or calcification. Although disc mineralisation has been detected in a range of animals, species differ in their propensity for this degenerative change, and equally the radiographic appearance of disc mineralisation is variable, dependent on both the location and integrity of the disc itself.[168; 190] Most often mineralised discs or disc material are detected in situ within the intervertebral disc space. Alternatively, either spikes of calcified material extending dorsally from the disc, or free calcified disc material within the vertebral canal, may be suggestive of disc extrusion or

protrusion. However calcified disc material can be difficult to visualise due to overlying anatomical structures, and differentiation from osteophytic reactions can be problematic.[168]

Consequently, when using plain radiography, indirect measures of disc degeneration are usually applied, including narrowing or collapse of the disc space, osteophytosis of vertebral body margins, and EP sclerosis and lucency.[159; 164; 189-191] Degenerative EP changes have recently received attention.[170] The CEP, as with articular cartilage of the synovial joint, cannot be directly assessed on radiography, and assessment of EP degeneration is confined to the osseous component. However, even for this structure, standard radiological approaches provide limited visualisation.[170] That said, radiographically detectable sclerosis of the osseous EP margins of the vertebrae are accepted signs of established disc degeneration.[164; 169] Whilst less common, EP lucency may also be detected. Previously, EP lucency in association with disc degeneration was considered to represent Schmorl's nodes. This finding has now been challenged, with radiographic EP lucency now postulated to represent a range of EP lesions and defects associated with disc degeneration, including but not exclusive to Schmorl's nodes.[170]

All indirect measures of disc degeneration are indicators of advanced disease, highlighting the inability of plain radiography to detect early degenerative changes within the disc. In addition, within advanced disease, it can be difficult to differentiate DDD in situ from that of disc displacement on plain radiography, particularly in the absence of disc mineralisation. Whilst some suggestive findings may be seen with disc displacement, including a reduced size and increased opacity of the intervertebral foramen, these changes can be both difficult to appreciate on plain radiography and difficult to attribute definitively to disc displacement.[159; 168]

However, plain radiography has shown utility for the detection of degenerative changes to other integral components of the intervertebral joint. Whilst facet OA may be difficult to detect in companion animals,[39] attention to correct positioning of a lateral spinal projection may show the typical changes of articular process remodeling with osteophytosis and thinning of the joint space.[159; 178] In contrast, spondylosis deformans is frequently detected on plain radiography, however radiographic appearance is dependent on stage and severity. Initially appearing as small, smooth, bony outgrowths adjacent to, but not extending beyond, the cranial and caudal

margins of the vertebral body, with progression the intervertebral space maybe bridged. Spondylosis deformans is commonly seen as one of a triad of radiographic changes that also includes narrowing of the joint space and EP sclerosis.[159]

Finally, whilst the spectrum of radiographic changes outlined above is highly suggestive of axial DJD, some radiographic features are not specific to this disease. Bridging spondylosis deformans must be differentiated from diffuse idiopathic skeletal hyperostosis (DISH), and clear guidelines have been established to facilitate discrimination between the two based on radiographic appearance.[178] Less easily differentiated on plain radiography are DDD and discospondylitis.[192] Whilst degenerative changes to the vertebral body, including radiographic lucency and shortening of the vertebral body, will facilitate a diagnosis of discospondylitis, other radiographic changes, namely collapse of the disc space, spondolytic reactions, and EP sclerosis and lucency, equally apply to DDD.[125; 168; 193] In particular, chronic resolved discospondylitis can be very difficult to differentiate from chronic DDD.[192]

There have been no systematic studies examining the radiographic appearance of axial DJD in captive large cats. The limited information available is derived from a small number of case studies of clinical IVDD[69; 88; 89] and spinal DJD [70] and a single case series of eight spinal-DJD affected large cats,[68] of which radiographs were available for six cases. With this caveat, the following observations are made. The case series provides the most comprehensive information, reporting narrowed or collapsed disc space(s) on radiography in all six animals, and associated spondylosis in all but one case. The only remaining radiographic change to be reported was the presence of mineralised discs, which were evident in two animals, one of which demonstrated multifocal disc mineralisation, with affected discs both in situ and dorsally displaced. Clinical case studies collectively described a similar picture, with both disc mineralisation and spondylosis reported in three of the four cases, narrowed intervertebral disc space reported in a single case, and one case reporting the additional findings of intervertebral foramen narrowing and collapse of the space between articular processes, considered suggestive of disc extrusion.[69] Of note, case reports included instances of both disc extrusion[69; 88] and protrusion.[89] In these cases, plain radiography detected both direct and indirect evidence of disc degeneration, with the demonstration of disc mineralisation, and narrowing of the intervertebral disc space and

intervertebral foramen respectively. However, in no case could plain radiography confirm a diagnosis of disc displacement, with the alternative diagnostic modalities of myelography, computed tomography and direct visualisation at surgery necessary for definitive diagnosis.

1.8 The clinical signs of DJD in captive large cats

1.8.1 The clinical signs of appendicular OA/DJD

The clinical signs of appendicular OA/DJD have been well researched in both human and companion animal medicine,[24; 34; 72; 112; 194; 195] and whilst there is some variation between species, commonalities exist. The clinical signs are widely acknowledged to be variable, dependent on a multitude of factors including joint site and stage of disease. Within individuals a spectrum of possibilities exists, from major disruption to subclinical disease. Clinical signs may fluctuate significantly with time,[30; 196; 197] with the natural course punctuated by periods of quiescence, stabilisation or progressive deterioration.[26; 40] Despite this variable presentation, several clinical signs are consistently reported: joint instability, pain, loss of function and mobility impairment,[24; 40; 194; 198] with the generation and role of pain in OA/DJD receiving particular attention.

In comparison, information regarding the clinical signs associated with OA/DJD of the appendicular skeleton of captive large cats is confined to only a small number of published case reports, containing minimal detail regarding clinical signs. Based on these limited studies, appendicular OA/DJD in captive large cats has been associated with lameness in some, but not all, cases. Lameness has been reported with coxofemoral, elbow and stifle OA, affecting tigers, cheetahs and snow leopards. Both unilateral, and bilateral disease have resulted in lameness,[43-45; 48; 89] and bilateral disease with unilateral lameness has also been noted.[93] Lameness has been reported in conjunction with a generalised decrease in activity levels,[44] and in the most extreme of cases, has resulted in euthanasia of the affected animal.[42]

Alternative clinical signs have also been reported. A tiger with coxofemoral OA, whilst not lame, demonstrated both stiffness and mobility impairment for a range of activities, including swimming, stretching up on the hind limbs, and moving on and off a raised bench.[43] Similarly, a cheetah affected with bilateral stifle DJD, whilst recording bilateral hindlimb lameness, also demonstrated reportedly atypical clinical signs of an abnormal ‘sagging’ hindlimb gait, and a

reluctance or difficulty rising.[45] However, the difficulty in assessment of all gait parameters, including lameness, general activity levels and specific actions, in a zoo setting has been noted. Whether this contributes to a delay or failure to recognise mobility issues is currently unclear.[43; 45]

OA/DJD has also been reported as a comorbidity, and in these cases any attributable clinical signs have proven difficult to discern. A cheetah with bilateral medial patellar luxation and associated OA presented with a long-standing history of bilateral hindlimb lameness, difficulty rising, and reluctance to walk. Whilst the clinical signs were not considered typical of patellar luxation in the dog or cat, it was not possible to determine the respective contributions of the two pathologies to the presenting signs.[45] OA/DJD has also been recognised as an incidental comorbidity. Unilateral coxofemoral DJD in a seven-year-old lion, presumed secondary to deformity of the acetabulum due to metabolic bone disease, was considered subclinical.[50] Similarly, a 13-year-old tiger diagnosed with multiple myeloma had bilateral stifle DJD identified opportunistically on necropsy. No mobility issues were described, and the finding was considered incidental.[49] Only one paper has reported behavioural changes associated with OA in captive large cats, with a failure to breed noted for a snow leopard with bilateral coxofemoral OA. The authors reported an improvement in behaviour and attitude after corrective surgery, however no causal association was established.[94]

Physical and orthopaedic examination findings from osteoarthritic large cats are similarly limited, with information confined to three case reports.[44-46] These papers, detailing coxofemoral and stifle OA, reported joint-associated abnormalities restricted to crepitus, reduced range of movement and atrophy of associated musculature. However, one of the three cases, involving OA of the stifle joints, had concurrent patella luxation,[45] and a second case was unable to definitively associate reduced range of motion of the stifle joint with any detectable impact on agility or activity.[46] As a result, the relevance to OA/DJD and clinical significance of these findings is debatable.

1.8.2 The clinical signs of axial DJD

The clinical signs of axial DJD, including pain, stiffness and those of associated intervertebral disc displacement (ambulatory changes and neurological deficits) have been extensively reviewed for companion animals.[32; 199; 200] In comparison, whilst axial DJD has been reported to frequently result in clinical signs in captive large cats,[41] the evidence for this statement is limited, with available information restricted to one case series [68] and a small number of case reports.[69; 70; 88; 89; 111]

The case series, a retrospective study of 37 large cats from the *Panthera* genus, reviewed clinical, radiography, and necropsy entries to identify degenerative spinal disease, defined as IVDD with or without spondylosis, in eight animals. The study reported that, although degenerative spinal disease was associated with clinical signs in large cats, the signs varied in their level of specificity for DJD. Progressive neurological clinical signs of hindlimb paresis and ataxia were highlighted, associated with severe hindlimb muscle atrophy. Gait abnormalities of lameness and stiffness were also reported. Clinical signs could be either acute, or chronic and intermittent with an insidious onset and a duration spanning months to years. Five of the eight animals were euthanased as a direct result of the neurological dysfunction due to their degenerative spinal disease.

Further detail regarding clinical signs at an individual case level is provided by five case reports, that combined described IVDD, including both disc protrusion and extrusion, and lumbosacral bridging spondylosis. Collectively, these case studies presented a comparable picture to that of the case series. Both acute [88; 111] and chronic [70] cases were described, with an additional case of peracute and severe progression of preexisting disease.[69] There was a similar focus on neurological involvement, with hindlimb ataxia, paresis, and paraplegia all reported. In addition, hindlimb proprioceptive deficits were identified in the case studies, variously described as toe dragging, crossing over of the feet, stumbling and knuckling.[69; 70; 89] An unusual manifestation of neurological pathology, self-mutilation of the hindlimbs, was also reported, however lameness was restricted to one case only.[70] Other evidence of mobility impairment was restricted to a single case that demonstrated both a reluctance to rise and a reluctance to move, associated

with intervertebral disc extrusion.[69] Although gait and proprioceptive abnormalities were detailed, as with appendicular OA/DJD case reports, reported findings from physical and orthopaedic examination were negligible, with only one entry of kyphosis,[89] and, in contrast to the case series, entries of hind limb muscle atrophy were conspicuously absent. The limited capacity to assess neurological impairment in these species was also noted.[88]

For both the case series and case studies, the reported clinical signs were overwhelmingly referable to the hind limbs. Whilst possibly a reflection of the high incidence of lumbar and lumbosacral involvement in large cats, this finding also pertained to the case of intervertebral disc extrusion in the caudal cervical spine,[89] with investigators noting the unusual sparing of forelimb involvement in presentation. Further research is indicated to clarify any preferential expression of mobility impairment to the hindquarters for these species.

Currently it is still difficult to determine the contribution of many of the different components of axial DJD in large cats, to the signs that they exhibit. This particularly pertains to disc mineralisation, collapsed disc spaces and spondylosis deformans. For example, whilst bridging spondylosis has been reportedly associated with a range of clinical signs, from an asymptomatic animal to severe neurological dysfunction,[185] this review was unable to determine the spectrum of clinical signs for this feature of axial DJD in large cats. This is likely due to the multifocal nature of degenerative spinal pathology, the reported association between spondylosis and IVDD, and low reported case numbers for axial DJD in these animals. Regardless, the clinical significance of these individual pathologies, if seen in isolation, is still unclear.

Finally, the 'nonspecific' clinical signs of decreased activity, weight loss and poor appetite have been reported in axial DJD-positive large cats, with these clinical signs particularly emphasised in findings from the case series.[68] Decreased activity has also been noted in geriatric captive jaguars with spinal disease,[77] and anorexia recorded for one of the five axial DJD positive case studies.[69] However, a decline in overall activity level and decreased appetite are recognised as common behavioral changes in ageing animals of a range of different species, including nondomestic felids.[75] Additionally, the case series did not report the incidence of these nonspecific signs in the DJD-negative cohort, nor was it clear whether comorbidities could have contributed to these

clinical signs. Consequently, the association between the expression of these nonspecific clinical signs and spinal DJD in large cats requires further clarification.

1.9 Radiography as a diagnostic tool for DJD

1.9.1 Advantages and Applications

Plain radiography remains the most widely implemented first-line diagnostic tool for the detection of OA/DJD, and spinal disease, including axial DJD, in both human and veterinary medicine.[33; 121; 164; 169; 201] Radiography has been validated as a diagnostic tool for the detection of both axial and appendicular DJD [119; 189; 202] and consequently has wide ranging applications, with roles in both clinical medicine and research.[121] Not only is radiography still considered the 'gold standard' for both diagnosis and staging of human OA,[119] but is also the initial imaging modality of choice for lower back pain in the human patient,[164] and for evaluation of spinal disease in general in companion animals.[168; 199; 201] Standardised plain radiography is used to monitor structural joint changes in osteoarthritic clinical drug trials [203] and for the domestic cat, retrospective radiological studies have proved integral for the identification of DJD as a disease of clinical significance.[35; 39; 64] These pioneering studies provided impetus for further prospective investigations, where DJD prevalence was found to be much higher than previously appreciated for this species.[36-38]

1.9.2 Limitations

The many limitations of plain radiography are wide ranging and beyond the scope of this review, however radiography presents several specific limitations when used for the detection of degenerative arthropathies of both the spine and synovial joints. Radiography cannot assess all joint structures, providing limited information only regarding soft tissue changes.[24; 34] In particular the disc and cartilaginous endplates of the intervertebral joint, and articular cartilage and synovium of the synovial joint cannot be visualised by radiography.[119; 189] Consequently, when structural degenerative changes are confined to these joint components, significant joint disease can occur in the absence of radiographically detectable change. This early or pre-radiographic DJD is a major contributor to the common observation that radiography has low sensitivity for DJD detection.[38; 167] Principally documented as a subcategory of OA, pre-

radiographic OA is recognised in human, companion animal and exotic animal medicine.[24; 26; 36; 65; 75; 119; 204] In addition, clinical disease may also result from molecular abnormalities that precede structural change to the articular cartilage, providing yet another explanation for pre-radiographic OA,[24; 26; 119] and further lowering radiographic sensitivity for DJD detection. In contrast, the radiographically detectable features of joint damage, structural change to the surrounding bony structures of the disc or articular cartilage, are indicators of late-stage disease.[30; 34; 189] For OA in particular, it is unclear how useful these radiographic features are for prediction of articular cartilage damage.[38]

Most radiographic signs of DJD are nonspecific, and the radiographic appearance of progressive joint disease will differ throughout the disease process.[139] Consequently, although radiography is useful for detecting later-stage DJD, it cannot always distinguish between different joint diseases, or different inciting causes, that result in similar pathology. Equally, as not all radiographic parameters lend themselves to objective assessment, a degree of subjectivity remains with interpretation of findings.[189] This is particularly the case when assessing severity of degenerative disease and represents a further limitation of radiography.

Finally, not only can painful DJD occur in the absence of radiographic changes, but, when detected, radiographic DJD may not necessarily represent clinically significant disease.[36; 65; 204] This incongruency between radiographic evidence and clinical impact is acknowledged for a range of species, including zoo mammals.[26; 40; 75; 185] Once again, this has been well considered for OA, where possible explanations include the complex and multifactorial pathogenesis of OA, the understanding that structural change does not necessarily equate with illness, and that a 'clinical threshold' must be surpassed for clinical signs to be expressed.[24; 37; 203]

1.10 Summary, and research aims and overview

1.10.1 Summary

As their population numbers in the wild decline, and conservation status remains at 'vulnerable' to 'endangered' levels, large cats are kept in increasing numbers in zoological institutions. Improvements in both veterinary care and husbandry have resulted in extended longevity, however there are many consequences of old age in captive large cats, including age-related diseases such as DJD. Whilst considered a disease with serious welfare implications, the current understanding of DJD in captive large cats is limited. To date there have been no large-scale studies investigating DJD in large cats, with current clinical information opportunistically sourced from individual animals only, and confined to a single case series of eight individuals affected by degenerative spinal disease, and a small selection of case studies detailing both axial and appendicular DJD.[43-45; 68-70; 88; 89] Combined these sources provide a limited picture of clinical presentation, radiographic appearance, and gross and microscopic pathology. Degenerative arthropathies of the axial skeleton are overrepresented in the literature, and there is a notable paucity of information regarding degenerative arthropathies in the cheetah. Supplementary information is provided by osteopathological studies. Whilst useful in characterising associated bony lesions, these studies have no capacity to evaluate either articular cartilage or the intervertebral disc, and demographic information and life history of study subjects are rarely available. All studies are further compromised by small sample sizes and potential bias as a result.

Consequently, even the most fundamental metrics for DJD in captive large cats, including prevalence, severity, causal factors and species susceptibility, are unknown. The utility of radiography as a diagnostic tool for DJD in these species is unproven, and whilst the clinical impact of DJD is considered significant,[41] there is little hard evidence to substantiate this position. As the correlation between radiographic evidence and clinical expression has not been explored, the clinical significance of radiographic DJD remains unclear.

1.10.2 Research Aims

Using radiographic studies and matched clinical records from 1979-2019, sourced from thirteen zoos and one referral veterinary teaching hospital from Australia, New Zealand and North America, the key aims of this PhD research were to:

1. determine the prevalence, distribution and severity of radiographic DJD in captive held cheetahs, lions and tigers;
2. characterise the radiographic features of DJD in these three species;
3. evaluate the association between radiographic DJD and a range of potential causal factors, including species, age, gender and, for cheetah, enclosure size;
4. gain further insight into the prevalence of both meniscal ossicles and the supinator sesamoid bone, and examine the association with DJD status of the stifle and elbow joints respectively;
5. determine a spectrum of DJD-associated presenting clinical signs for radiographic DJD in captive cheetahs, lions and tigers, and examine the correlation between the recording of these clinical signs at presentation for imaging, and a range of variables including radiographic DJD status, non-DJD morbidities, species, age and gender.

1.10.3 Research overview

Chapter 1 presents a review of the literature relating to DJD in both captive and free-ranging large cats and reviews the pathogenesis of osteoarthritis and degenerative disease of the intervertebral joint with a focus on intervertebral disc degeneration.

Chapter 2 presents the findings of a radiological investigation into arthropathies of the axial skeleton in captive-held lions, tigers, and cheetahs. The prevalence, distribution and severity of all intervertebral joint disease was determined and the radiographic features described. The association between joint disease and a range of predictors was examined, and the most frequently, and severely affected axial segments identified.

Chapter 3 presents the findings of a radiological investigation of arthropathies affecting the six major synovial joint types of the appendicular skeleton, for captive-held cheetahs, lions and

tigers. The prevalence, distribution and severity of appendicular joint disease was determined and the radiographic features described. The association between joint disease and a range of predictors was examined, and the most frequently and severely affected appendicular joint identified, for each of the three species.

Chapter 4 presents a radiological investigation of joint disease across the total skeleton, with the likelihood for overall arthropathy detection in the large cat patient determined and significant associations between potential risk factors and arthropathies reported. Joint disease in the axial skeleton was compared with that for the appendicular skeleton, with particular emphasis on inter-species differences.

Chapter 5 presents an investigation of two radiographically detectable, joint-associated skeletal structures, the meniscal ossicle of the stifle and the supinator sesamoid bone of the elbow. The prevalence of both structures was reported, and the association with radiographic DJD status discussed.

Chapter 6 presents an investigation of the association between DJD-associated clinical signs as observed in companion animals, and a range of predictors, including radiographic DJD, for all cheetahs, lions and tigers that underwent radiography during the data acquisition period of 1979-2019. Utilising clinical signs entries recorded at presentation for imaging, a broad, highly sensitive screening analysis was conducted, and a spectrum of DJD-associated clinical signs for captive large cats was identified.

Chapter 7 summarises the key findings from this thesis and identifies and prioritises areas for future research based on these findings. The many limitations inherent with retrospective studies are addressed, in addition to the challenges provided by wildlife research.

Appendix 1.1 Census of nondomestic felid species held globally, as of 16th April, 2020; Species360 (ZIMS), with associated conservation status (IUCN).

Felid Species Common name	***Scientific name***	**IUCN Red List status** [205]	**Number of living held in captivity** [13]
Lion	***Panthera leo***	**Vulnerable**	**2484**
Tiger	***Panthera tigris***	**Endangered**	**1827**
Snow leopard	*Panthera uncia*	Vulnerable	410
Jaguar	*Panthera onca*	Near Threatened	415
Leopard	*Panthera pardus*	Vulnerable	959
Cheetah	***Acinonyx jubatus***	**Vulnerable**	**1384**
Mainland clouded leopard	*Neofelis nebulosa*	Vulnerable	283
Sunda clouded leopard	*Neofelis diardi*	Vulnerable	6
Serval	*Leptailurus serval*	Least Concern	507
African golden cat	*Caracal aurata*	Vulnerable	0
Caracal	*Caracal caracal*	Least Concern	211
Andean cat	*Leopardus jacobita*	Endangered	0
Ocelot	*Leopardus pardalis*	Least Concern	234
Margay	*Leopardus wiedii*	Near Threatened	84
Pampas cat	*Leopardus colocola*	Near Threatened	0
Southern tiger cat/tigrine	*Leopardus guttulus*	Vulnerable	10
Northern tiger cat/tigrine	*Leopardus tigrinus*	Vulnerable	44
Geoffroy's cat	*Leopardus geoffroyi*	Least Concern	72
Guina or Kodkod	*Leopardus guigna*	Vulnerable	0
Bobcat	*Lynx rufus*	Least Concern	301
Canada lynx	*Lynx canadensis*	Least Concern	129
Eurasian lynx	*Lynx lynx*	Least Concern	536
Iberian lynx	*Lynx pardinus*	Endangered	11
Marbled cat	*Pardofelis marmorata*	Near Threatened	9
Borneo bay cat	*Catopuma badia*	Endangered	0
Asiatic golden cat	*Catopuma temminckii*	Near Threatened	35
Jaguarundi	*Herpailurus.yagouaroundi*	Least Concern	95
Cougar	*Puma concolor*	Least Concern	423
Rusty-spotted cat	*Prionailurus rubiginosus*	Vulnerable	55
Flat-headed cat	*Prionailurus planiceps*	Endangered	9
Fishing cat	*Prionailurus viverrinus*	Vulnerable	206
Leopard cat	*Prionailurus bengalensis*	Least Concern	394
Pallas's cat	*Otocolobus manul*	Near Threatened	173
Jungle cat	*Felis chaus*	Least Concern	185
Black-footed cat	*Felis nigripes*	Vulnerable	44
Sand cat	*Felis margarita*	Least Concern	171
Chinese mountain cat	*Felis bieti*	Vulnerable	0
Wild cat	*Felis silvestris*	Least Concern	261
Grand Total			**11967**

Appendix 1.2 Age ranges (years) for mean and maximum age at death, reported for captive-held and free-ranging cheetah, lions and tigers.

	Age at death			
	Captive (years)		Free-Ranging (years)	
	Mean [19]	Maximum [18; 19; 51; 85; 86]	Mean [20; 47; 206]	Maximum [20; 47; 105; 206-208]
Cheetah	11.2	11-19.1	5.3-6	9.3 to 15
Lion	15.5	25.2 to 30	6.5	14.2 to 29
Tiger	15.7	21.4 to 26	not available	7-26

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Chapter 2

A retrospective radiological study of arthropathies of captive lions, tigers, and cheetahs

Part I: The Axial Skeleton

2.1 Introduction

Large nondomestic felids are commonly held in zoological institutions, living an estimated 25% longer in captivity than the wild. With longevity comes an increased incidence of diseases of chronicity and advancing age, raising both medical concerns and welfare issues.[1] Whilst it is acknowledged that degenerative joint disease (DJD) of both the axial and appendicular skeleton features amongst these age-related diseases,[2] there is scarce information in peer-reviewed published literature regarding the prevalence, radiographic features, risk factors for, and impact of, DJD in large cats. Additionally, available studies examining axial DJD are confined to a singular case series detailing radiographic, necropsy and clinical record findings of eight members of the genus *Panthera*,[3] and a small number of clinical case studies reporting degenerative spinal disease and its sequelae in the tiger[4-8] and the lion.[9] There are no studies to date reporting axial DJD in the cheetah.

This retrospective study was undertaken to address the knowledge gaps in the current understanding of the radiographic prevalence, distribution, severity and features of axial arthropathies of lions, tigers, and cheetahs. Further, in order to examine potential risk factors for the development of axial DJD, associations between radiographic axial DJD status, and a range of animal demographics (species, age, gender) were examined. An additional analysis of the impact of enclosure size on axial arthropathies in the cheetah was also included.

2.2. Materials and Methods

2.2.1 Data acquisition

Thirteen zoos and one referral veterinary teaching hospital from Australia, New Zealand and North America provided case material for this study. Of the participating zoos, nine were classified as urban and four open-range. The clinical records and radiographic studies of all lions, tigers and cheetahs for the years 1979-2019 were accessed, resulting in the identification of 702 radiographic studies from 305 animals.

All animal studies were then assessed for suitability for inclusion. Animals had to be six months or older at the time of radiographic examination, with diagnostic images of the axial skeleton available for review, in order to be included in the study. For individual radiographs, the skeletal anatomy of one or more intervertebral joints needed to visualised in entirety for the image, and corresponding radiographic study, to be assessed as eligible for inclusion. A total of 231 individuals met the above inclusion criteria. These animals collectively generated 469 radiographic studies containing 1956 images of the axial skeleton.

2.2.2 Age Class Classification

Animals were categorised into four age classes: young adult, adult, senior and geriatric. Age classification was based on species reproductive parameters and total life spans of these captive populations, with 'Young Adult' representing those under or approaching reproductive age, 'Adults' of reproductive age, 'Senior' of an age at or beyond reproductive senescence and 'Geriatric' representing those animals of an age beyond what is typically encountered in free ranging counterparts.[1; 10-13] Consequently, age range within class differed between the cheetah versus the lion and tiger: Young Adult (cheetah: 6months to < 4 years, lion/tiger: 6months to <5 years), Adult (cheetah: 4 to <9 years, lion/tiger: 5 to <12 years), Senior (cheetah: 9- < 12 years, lion/tiger: 12 to <16 years), Geriatric (cheetah: ≥12 y, lion/tiger: ≥ 16 years).

2.2.3 Radiological scoring system design and application

A study-specific radiology scoring system modified from protocols previously described for domestic cats was developed.[14; 15] The axial skeleton was divided into cervical, thoracic,

thoracolumbar junction, lumbar and lumbosacral junction segments, and sacroiliac joints. The number of intervertebral joints captured was recorded and, where possible, the anatomical location of an abnormality noted. Inter- and intraspecies variation of the thoracic anticlinal vertebra rendered difficulty in ascribing an exact anatomical location for pathology in some of the thoracic studies. In these cases, the terms cranial, mid or caudal thoracic were substituted for vertebral formula. Intervertebral joints (intervertebral disc spaces and articular facets) were assessed individually within segments, and assessed for the presence of the following features of arthropathy: spondylosis deformans (0 absent, 1 present without bridging, 2 present with bridging), intervertebral disc space narrowing (0 absent, 1 present), intervertebral disc mineralisation (number and site(s) recorded), vertebral endplate sclerosis and lucency (0 absent, 1 present), and osteophytosis of sacroiliac and articular facet joints (0 absent, 1 mild, 2 moderate, 3 severe). Any site with an intraarticular fracture was also recorded. Each axial segment was then assigned an arthropathy score of negative or positive, with a positive score denoting one or more intervertebral joints showing feature(s) of arthropathy. A subjective severity score of mild, moderate or severe was then assigned to every arthropathy-positive segment. If a study included one or more arthropathy-positive axial segments, this study was assigned a positive arthropathy score for the axial skeleton.

All radiographic images were scored by the primary author (LB). A validation process was undertaken, with the initial 44 of 469 (9.4%) radiographic studies also reviewed and scored by a board-certified DACVR radiologist, Dr. Alex Young(AY). Subsequently, where lesions were equivocal, images were reviewed by both observers together and a consensus was reached. As a result, of the 469 studies eligible for inclusion, 104 (22.2%) of all studies were reviewed by both observers. Archived radiographic film were read and scored on site or, where indicated, digitised for subsequent reevaluation. Digital radiographic studies were viewed using a DICOM-viewing software (eFilm 3.1, Merge Healthcare, Milwaukee, MI, or RadiAnt® 2020.1 and earlier versions of DICOM-viewing software) on high-resolution colour computer monitors. Both reviewers were blinded to any clinical information regarding the study at the time of radiological scoring.

For every radiographic study, animal identification data (global accession number, species, subspecies, gender, date of birth), date of imaging study, age at time of imaging in months and

age class, institution where the animal was held at the time of the study with corresponding local identification number and house name, the number of images (total and number of readable images) and review by the second observer (yes/no) was recorded in a spreadsheet (Microsoft ® Excel 2016) database. References for normal and pathological features of the axial skeleton for the study species were determined by reviewing anatomical and radiographic texts for domestic animals,[16; 17] publications on normal anatomy of the axial skeleton of large cats,[18-21] and examination of osteopathological specimens of captive cheetahs, lions and tigers, held at the School of Animal and Veterinary Sciences, University of Adelaide.

2.2.4 Determination of nature of arthropathy

Although there are certain radiographic features that are considered typical of degenerative joint pathologies, it can be difficult to differentiate degenerative disease using radiology alone. For this reason, patient history, and physical examination findings from matched clinical records were used, where available, to assist categorisation of arthropathies into degenerative versus nondegenerative groupings. This was conducted after radiographs were assessed. Nondegenerative arthropathies were further classified as either suspected or confirmed developmental, traumatic, septic or of unknown aetiology. Degenerative arthropathies were further classified as either a result of developmental disease, trauma, or sepsis, or of no inciting cause identified. Arthropathies that transitioned from a nondegenerative aetiology to a degenerative joint disease at a future date within the study period, were also identified.

2.2.5 Data preparation for analysis

Some animals had their axial skeletons imaged on multiple occasions across multiple age classes, resulting in duplicate studies or repeat measures. To keep from biasing the data, when animals had multiple studies in an age class, only one study per age class (henceforth referred to as 'animal within an age class') was included. A study was considered duplicate for an individual if it captured the same skeletal level within the same age class. The protocol for determination of which studies to include and which to remove, within age class, was based on arthropathy status from radiographic scoring, and chronological order. If all studies were negative for arthropathy, the most recent study was included. If all studies were arthropathy positive, the earliest study

was included. Where arthropathy status transitioned from negative to positive within age class, the chronologically first arthropathy-positive study was included in the data set. Where there were was an insufficient number of studies for a particular analysis to be divided into age classes, the age classes were pooled. This was necessary for analyses of the most severely affected axial segment and the prevalence of features of axial arthropathies. Here, each animal was restricted to one entry per data set. For those animals imaged on multiple occasions, scoring information from the most recent study was included.

2.2.6 Statistical Analysis

The radiologic scoring data was analysed at two levels, the spine as a single unit and the six axial segments independently. Both inferential and descriptive analyses were performed, dependent on the number of observations within the dataset and subsequent power of the study.

2.2.6.1 Inferential Statistics

Logistic regression via a generalised linear mixed model with an underlying binomial distribution (GenStat, Version17, VSNi) was used to explore the association between the arthropathy status (negative, positive) for the axial skeleton, and the following predictors: species, gender, age class, and interactions between these three variables. All modelling was performed at an age class level, with animal identification included as a random effect. In addition, due to the high number of cheetahs in the study, and their dispersal between open range and urban zoos, the effect of enclosure size on the prevalence of cheetah arthropathies was also investigated. Due to the weighting of cheetah numbers for one open-range institution (Institution A), modelling was performed for both urban versus open range, and Institution A versus all other zoos. Interactions between cheetah enclosure size, age, and gender were also modelled. Due to smaller data sets for some individual axial segments (there were only 86 studies for the cervical spine), a generalised linear model (GenStat, Version17, VSNi) was used for logistic regression analysis of the association between the above predictors and arthropathy status (negative, positive) at an axial segment level. As with the generalised linear mixed model, modelling was performed at an age class level.

Modelling was based on a binary output for arthropathy status (negative or positive). All predictors were categorical, and either nominal with several categories (species) nominal and dichotomous (gender, cheetah enclosure size) or ordinal (age class). The level of statistical significance for association between arthropathy status and potential predictors was set at $P<0.05$. Due to small data set size for some analyses, a more liberal P-value range of ≥ 0.05 and <0.1 was considered trending to significance. Predicted means with associated standard errors were generated for all significant associations. Where indicated, least significant differences (LSDs), either manually calculated or computer-software generated (GenStat, Version17, VSNi), were used to perform pairwise comparisons of predicted means for subcategories within significant predictors.

2.2.6.2 Descriptive Statistics

Descriptive statistics were used to examine the prevalence, maximum severity, distribution patterns, features and nature of axial arthropathies. The majority of descriptive analyses were conducted according to age class, however age classes were pooled for smaller data sets. Pivot tables were utilised to generate tabulations, and where indicated, graphical depictions generated to illustrate key findings.

The severity of arthropathies was analysed at an axial segment level and for the spine in toto. By utilising the severity score generated for each arthropathy-positive segment (described previously), the most severely affected axial segment for each of the three species was determined. The analysis of the severity of joint disease for the spine in toto necessitated a single severity score to be assigned to every study, irrespective of the number of segments captured. The methodology used was to consider the severity scoring for all axial segments captured. The highest severity score achieved at an individual axial segment level was then ascribed to the spine in toto, becoming the final severity score for the spine for that study. This then allowed a comparison of severity between the four age classes within species, and between the three species.

Unifocal versus multifocal distribution of arthropathies within the axial skeleton was investigated for all arthropathy-positive animals that underwent imaging of more than one axial segment

within an age class. Distribution considered the axial segment, rather than the intervertebral joint, as the level of interest. Unifocal disease was therefore considered disease confined to a single axial segment, as opposed to a single intervertebral joint, and cases with multiple intervertebral joints affected within the one axial segment were scored as unifocal. A multifocal distribution was considered disease encompassing more than one axial segment.

Investigation of the prevalence of features of axial arthropathies was conducted on data sets where age classes were pooled, with results representing an aggregated score from all radiographic findings for that axial segment over the life of the animal. Features of axial arthropathies analysed were bridging spondylosis deformans, narrowed or collapsed intervertebral disc space, intervertebral disc mineralisation, facet osteoarthritis (OA) and endplate sclerosis and lucency. Each feature was scored as either present or absent, with a severity score of 1-3 recorded for facet OA. For every arthropathy-positive cervical, thoracic and lumbar segment, counts were made of the number of intervertebral joints captured and number with pathological changes, including separate counts of the number and location of bridged intervertebral joints.

2.3 Results

2.3.1 Study Population

A total of 313 radiographic studies of 'animals within an age class', representing 231 individual animals (Figure 2.1), were analysed for the prevalence of arthropathy in the axial skeleton. Seventy five of 313 (24.0%) studies were positive for arthropathy at one or more axial segments. The frequency distribution of modelled predictors against axial arthropathy status is presented in Appendix 2.1.

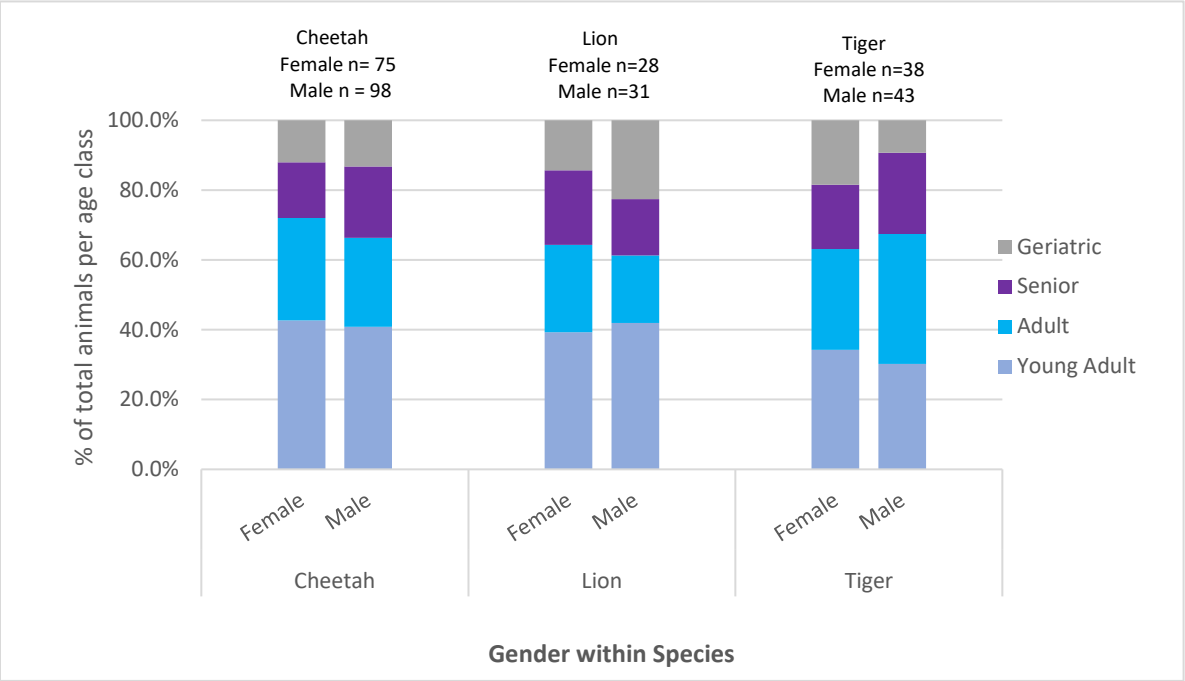

Figure 2.1 Distribution of 'animal within an age class' study population for analysis of arthropathies of the axial skeleton. n= number of individuals, gender within species

2.3.2 Modelling for predictors of arthropathy status

The most significant predictor for arthropathies of the spine was increasing age (P<0.001), with the predicted prevalence of axial arthropathies in young adult to geriatric age classes 7.8%, 22.1%, 29.8% and 59.0 % respectively. Pairwise comparisons showed that the prevalence of axial arthropathies in geriatric animals were significantly higher, and young adults significantly lower, than all other age classes, with no difference between the adult and senior age classes (Figure 2.2). The only other significant predictor was enclosure size in the cheetah subset (P=0.008), with

cheetahs held at Institution A predicted to have a significantly lower prevalence of axial arthropathies than those held at all other institutions (9.0% versus 26.7% respectively). Open range versus urban institution analysis trended towards significance (P=0.067), with predicted axial arthropathy prevalence of 13.5% versus 27.6% respectively. P values from all modelling can be found in Appendix 2.2. Appendix 2.3 shows the predicted means (non back transformed means), and standard errors for the differences, used for manual calculations of pair wise (LSD) calculations.

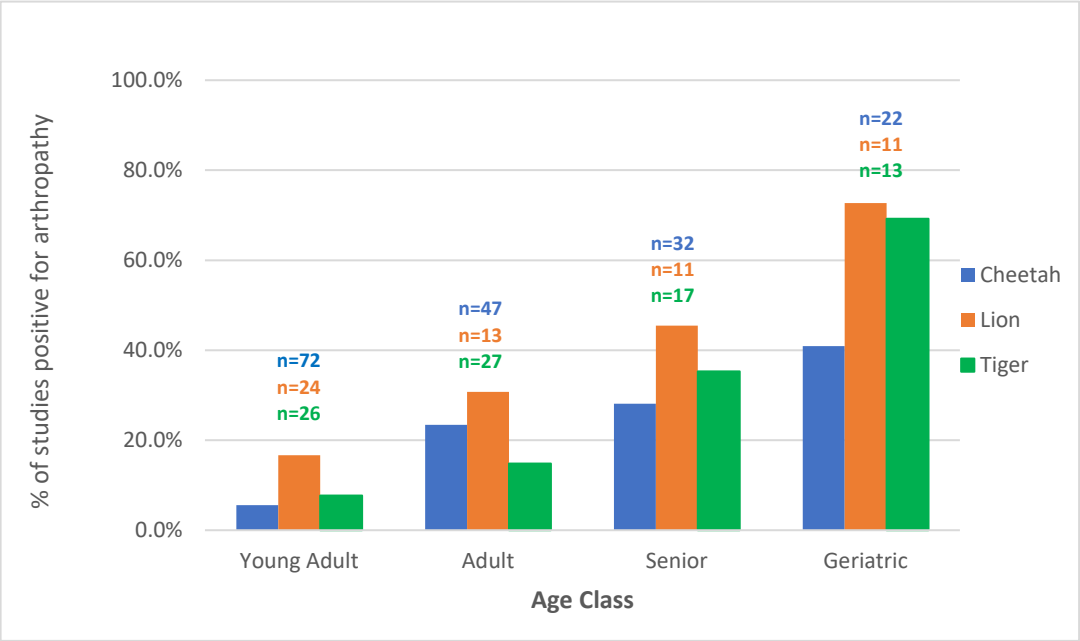

Figure 2.2 Distribution of the observed prevalence of arthropathies of the axial skeleton for cheetahs, lions and tigers, as a function of age. n= number of cheetahs/lions/tigers in an age class

The observed prevalence of arthropathies at an axial segment level, for 'animal within an age class' are presented in Table 2.1, with the frequency distribution of modelled predictors against arthropathy status at an axial segment level presented in Appendix 2.4 a-e. Modelling for the association between enclosure size and arthropathies in the cheetah at the axial segment level was restricted to thoracic and lumbar segments due to an absence of intervertebral joint disease in the cheetah at any other axial segment level.

Segment Level	Total number	Percentage arthropathy positive
C1-T1	86	12.8%
T1-13	209	12.4%
Thoracolumbar junction	195	3.1%
L1-7	262	17.2%
Lumbosacral junction	219	5.5%
Sacroiliac joint	142	9.9%

Table 2.1 Prevalence of axial segment arthropathies for 'animal within age class', three species combined.

The only significant predictor for arthropathies of the cervical spine was age class. Increasing age was significantly associated with the prevalence of intervertebral joint disease at the C1-T1 axial segment (P= 0.024) with pairwise differences showing that the geriatric age class had significantly higher predicted prevalence (66.7%) than all other age classes. This axial segment had the smallest data set across the vertebral column and the study may have been underpowered below the level of detection for all significant predictors.

There was an association between arthropathies of the thoracic spine and both species (P<0.001) and age (P=0.011). Tigers had a significantly higher predicted prevalence of arthropathy (29.8%) compared with cheetahs (5.3%). Young adults had a significantly lower predicted prevalence (0%) than all other age classes. The predicted prevalence for adults was lower than senior or geriatric animals (8.1%), however there was no significant difference between senior and geriatric large cats.

Age class (P <0.001) and gender (P=0.024) were significantly associated with arthropathies of the lumbar spine. The mean predicted prevalence for males was significantly higher than females (21.9% versus 11.3% respectively), and pairwise comparisons showed that young adults were predicted to have significantly lower (5.2%) and geriatric animals significantly higher (42.5%)

prevalence of lumbar arthropathies, with no difference between adult and senior age classes. In addition, the lumbar spine was the only axial segment where a significant association between enclosure size and arthropathy was demonstrated for the cheetah. Cheetahs held at Institution A were significantly less likely to show lumbar disease than those held at other institutions (P=0.002, predicted prevalence 7.7% versus 28.4% respectively). This association was repeatable, though only trending to significance (P=0.080) when all open-range cheetahs were compared with those from urban institutions.

There was a species effect in both the lumbosacral junction (P=0.016) and sacroiliac joint (P=0.003), with lions showing a significantly higher excepted prevalence of arthropathies (15.4%) than cheetahs (0.1%) in the lumbosacral junction, and both tigers and cheetahs in the sacroiliac joint. There was also a gender association in the sacroiliac joint (P=0.023), with females predicted to have a significantly higher arthropathy prevalence than males (16.2% versus 4.1% respectively).

No significant association was found between thoracolumbar junction arthropathies and examined predictors. P values for the modelling of associations between predictors and arthropathies at six axial segment levels are shown (Appendix 2.5). All results for significant associations between arthropathy at an axial segment level and modelled predictors are presented in Appendix 2.6 a-e.

2.3.3 The most frequently affected axial segment

A total of 1,113 axial segments from the three species across four age classes were scored for arthropathy status. With the exception of the thoracolumbar junction in young adult lions, the lumbar spine with or without the adjacent lumbosacral junction was the most frequently observed axial segment on the reviewed radiographs, in each of the four age classes, for all three species. Of the 1,113 segments, 114 individual segments, or 10.2% of all segments evaluated, showed radiographic features of arthropathy. The frequency of arthropathies at an axial segment level, for animals within age class, for the three species is shown (Appendix 2.7 a-c).

The lumbar spine was the most frequently affected axial segment for the cheetah across all age classes (Figure 2.3a). The frequency of lumbar disease was lowest in young adults (4/62, 6.5%), similar between adult (10/42, 23.8%) and senior (6/27, 22.2%) age classes, and greatest for geriatric (7/21, 33.3%) cheetahs. The sacroiliac joint was the most frequently affected axial segment for young adult, adult and senior lions (Figure 2.3b) however for the geriatric age class, the most frequently affected axial segments were the cervical and thoracic spine (Figure 2.3c), with 75% of all lions imaged showing disease. The prevalence of sacroiliac disease also remained high for this age class, with 67% of all sacroiliac joints of geriatric lions showing radiographic arthropathy. Sacroiliac joint disease was consistent with OA of the sacroiliac joint, typically confined to mild to moderate osteophytosis. In addition, two cases of fusion of the sacroiliac joint were detected. The prevalence of radiographic arthropathies of the axial segments of tigers increased with age. The segment most frequently affected was the cervical spine in the geriatric age class (4/4, 100%), followed by the thoracic (7/9, 77.8%) and lumbar 97/10, 70%) spines, also in this age class. The thoracic spine was the most frequently affected segment for the senior tiger. For the three species, when animals were pooled for age class, the most frequently affected axial segment in cheetahs remained the lumbar spine, for lions the sacroiliac joint, but for tigers the thoracic spine was the most frequently affected (Figure 2.4).

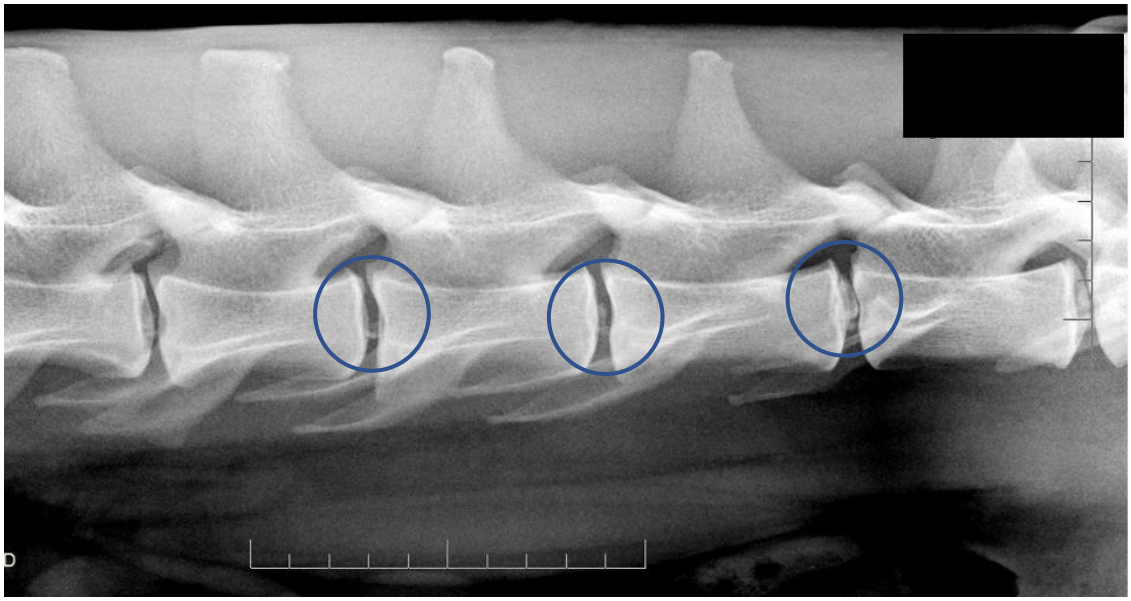

Figure 2.3a Lateral radiograph of the lumbar spine of an adult (6 y) male cheetah showing multiple (circled) in situ mineralised intervertebral discs.

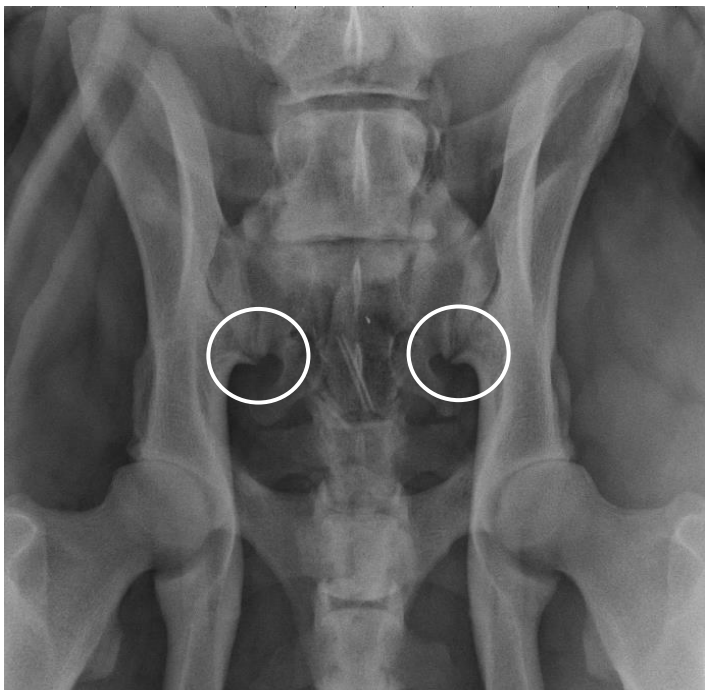

Figure 2.3b Ventrodorsal projection of the pelvis of a geriatric (17 y) lioness showing bilateral sacroiliac joint osteophytosis (circles).

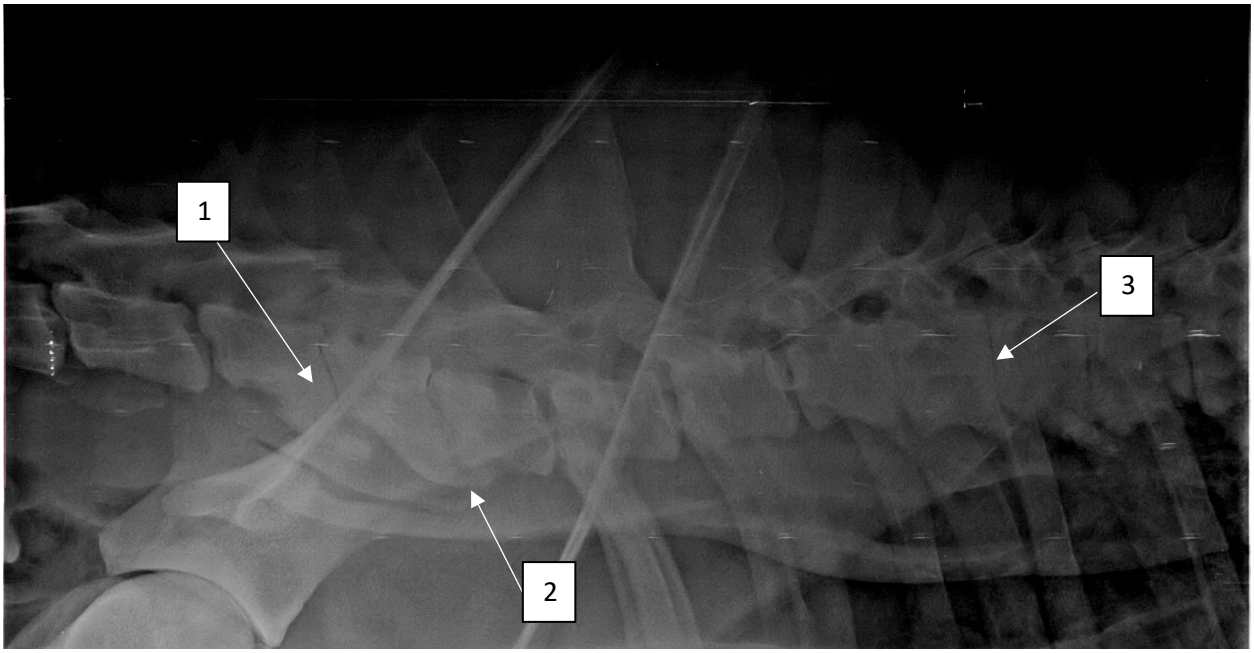

Figure 2.3c Radiograph of a severly affected cervico-thoracic spine of a senior (16 y) male African lion showing endplate sclerosis (1), bridging spondylosis deformans (2) and intervertebral space collapse (3).

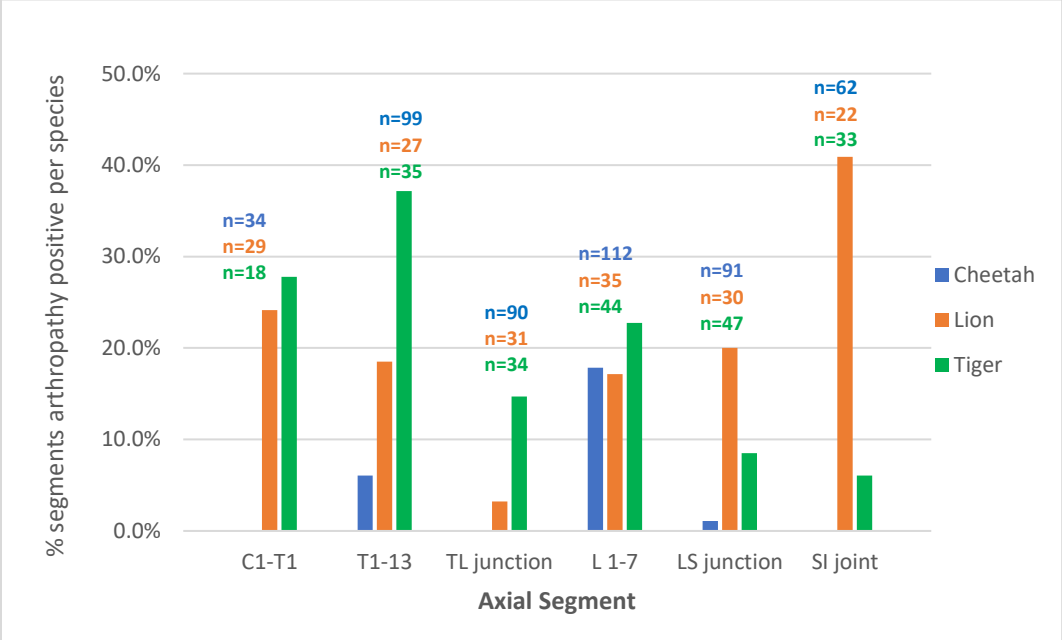

Figure 2.4 Frequency of arthropathies at axial segment level for the three species, age classes pooled. n= number of cheetahs/lions/tigers

2.3.4 Severity of axial arthropathies

For cheetahs, although the lumbosacral junction had the highest average maximum severity, only one individual recorded an arthropathy at this site. More representative of the data was the finding that the thoracic segment was the most severely affected segment (severity score 1.7, mild-moderate), closely followed by the lumbar spine. In addition, the severity for cheetah axial segment arthropathies was greater for those held at urban institutions (1.9) compared with those in open range facilities (1.2). The most severely affected axial segment in the lion was the cervical spine (Figure 2.3c) with an average severity of 2.5 (moderate-severe). The cervical spine was also the most severely affected segment for the tiger with a severity score of 2.8 (moderate-severe). All axial segments of the tiger, with the exception of the sacroiliac joint, had severity scores of ≥2 (moderate-severe). As such, tigers were found to have on average the most severe axial disease of the three species. Tabulations of severity data at an axial segment level for each of the three species are presented in Appendix 2.8 a-d.

To examine the effect of age, the severity of axial arthropathies was also examined for the four age classes, at the level of the axial skeleton (Figure 2.5). The maximum severity recorded in the axial skeleton differed between age class and species, with the most severe disease shown by tigers in the geriatric age class.

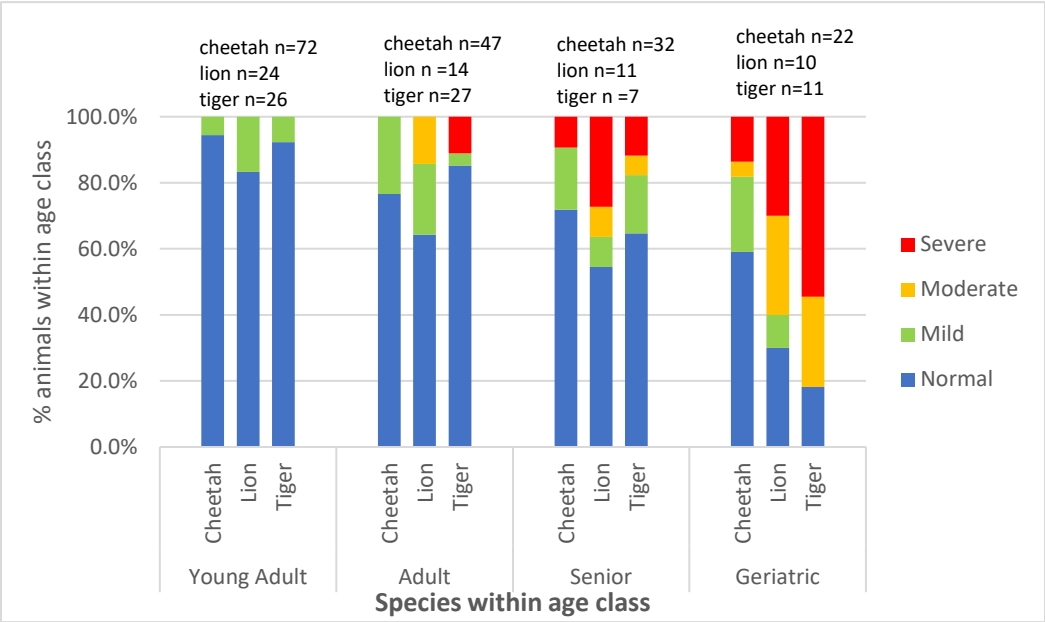

Figure 2.5 Distribution of maximum severity of arthropathy of the axial skeleton for three species across four age classes. n= the number of individuals in an age class.

In addition, in order to highlight those segments where disease and severity is most likely to occur, a comparison of prevalence and severity of disease, between each of the six axial segments, was also investigated (Table 2.2). Conducted at the level of age class within species, results highlighted the preponderance for severe cervical disease in older lions and tigers, and the generally widespread distribution of disease in older individuals of both species. In contrast, the vastly different disease pattern in the cheetah, of a low prevalence of moderate pathology confined to the lumbar spine, is illustrated.

		C1-T1	T1-13	Thoraco-lumbar junction	L1-7	Lumbo-sacral junction	Sacroiliac joint
Cheetah	Young Adult						
	Adult						
	Senior						
	Geriatric				X		
Lion	Young Adult						X
	Adult						X
	Senior	XX	X	X	X	X	XXX
	Geriatric	XXX	XXX		X	X	XX
Tiger	Young Adult						
	Adult						
	Senior	X	XX				
	Geriatric	XXX	XXX	XX	XX	X	

Table 2.2 Prevalence and severity of arthropathies at an axial segment level for the lion, tiger and cheetah. Prevalence : X ≥ 25%, < 50%; XX ≥50%, <75% ; XXX ≥ 75%. Severity: Green, mild, average severity 1.0-1.5; Yellow, moderate, average severity score 1.6-2.4; Red, severe, average severity score ≥ 2.5.

2.3.5 Features of Axial Arthropathies

Intervertebral disc space narrowing or collapse was the most common axial arthropathy feature recorded across the three species (64/90, 71.1% of axial segments recorded this feature), followed by endplate sclerosis (37/90, 41.57%). Of the 24 recordings of disc mineralisation within an axial segment, there were only three observations of disc material displaced dorsally into the spinal canal. All recordings of endplate lucency were seen in association with endplate sclerosis, and all endplate changes were seen in association with narrowing or collapse of the corresponding intervertebral disc space.

Species differed with respect to the relative frequencies of the different features, and their distribution across the spine. Intervertebral disc mineralisation was common in the lumbar spine of the cheetah (15/20, 75%). Disc mineralisation was also detected in six of 24 lion axial segments, with mineralisation confined to the thoracic spine and thoracolumbar junction. In contrast, in the tiger only three of 39 axial segments demonstrated any radiographic evidence of intervertebral disc mineralisation. Facet joint OA was detected in the tiger (7/39, 18.0% of all segments) and

cheetah (3/27, 11.1% of all segments) but not the lion. Eighty percent of all facet OA was graded as mild-moderate. Bridging spondylosis was rare in the cheetah (1/27, 3.7% of all segments) compared with the lion (8/24, 33.3% of all segments) and tiger (24/39, 61.5% of all segments). All arthropathy-positive lumbosacral junctions, and 80% of affected cervical segments, showed bridging spondylosis in the tiger. There were 11 examples from 9 animals (3 lions, 6 tigers) of multiple (≥3) contiguous bridged vertebrae. The caudal cervical spine and cranial lumbar region were most likely to display this pattern, with six of the 11 (54.5%) fused areas associated with degeneration of the intervertebral joint immediately cranial to the ankylosed region.

The most commonly observed features for cheetah were intervertebral space narrowing or collapse in the thoracic spine and intervertebral disc mineralisation of the lumbar spine (Figure 2.3a). For the lion, the highest prevalence of features was recorded for the cervical spine. All cervical segments showed intervertebral space collapse, with four of six (66.7%) showing accompanying endplate sclerosis. Lucency of the endplate accompanied sclerosis in 75% of affected cervical segments. Endplate sclerosis and lucency were also a feature of cervical and to a lesser extent thoracolumbar and lumbosacral disease in the tiger. Disease of the endplate was less frequently observed in the cheetah spine and confined to isolated cases in the thoracic and lumbar spine. Except for intervertebral disc mineralisation, tigers showed the highest prevalence of all axial features under investigation (Figure 2.6), with changes recorded for all spinal levels. Tabulations for the prevalence of selected features of axial arthropathies, at an axial segment level, for the three species, with age classes pooled, are presented in Table 2.3a-b.

Species	Axial Segment	Total	bridging spondylosis deformans		intervertebral disc space narrowing/collapse		intervertebral disc mineralisation		endplate sclerosis		endplate lucency		facet joint OA	
Cheetah	T1-13	6	0	0.0%	6	**100.0%**	0	0.0%	2	33.3%	1	16.7%	0	0.0%
	L1-7	20	1	5.0%	5	25.0%	15	**75.0%**	3	15.0%	2	10.0%	3	15.0%
	Lumbosacral junction	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Total		**27**	**1**	**3.7%**	**11**	40.7%	**15**	**55.6%**	**5**	**18.5%**	**3**	**11.1%**	**3**	**11.1%**
Lion	C1-T1	6	3	50.0%	6	**100.0%**	0	0.0%	4	**66.7%**	3	**50.0%**	0	0.0%
	T1-T13	5	2	40.0%	5	**100.0%**	2	40.0%	2	40.0%	1	20.0%	0	0.0%
	Thoracolumbar junction	1	0	0.0%	0	0.0%	1	100.0%	0	0.0%	0	0.0%	0	0.0%
	L1-L7	6	0	0.0%	3	50.0%	3	**50.0%**	2	33.3%	0	0.0%	0	0.0%
	Lumbosacral junction	6	3	50.0%	5	**83.3%**	0	0.0%	2	33.3%	1	16.7%	0	0.0%
Total		**24**	**8**	**33.3%**	**19**	79.2%	6	**25.0%**	**10**	**41.7%**	**5**	**20.8%**	**0**	**0.0%**
Tiger	C1-T1	5	4	**80.0%**	5	**100.0%**	0	0.0%	5	**100.0%**	5	**100.0%**	2	**40.0%**
	T1-T13	13	5	38.5%	13	**100.0%**	0	0.0%	5	38.5%	3	23.1%	1	7.7%
	Thoracolumbar junction	5	3	60.0%	5	**100.0%**	0	0.0%	4	**80.0%**	2	40.0%	0	0.0%
	L1-L7	11	7	63.6%	7	63.6%	3	27.3%	5	45.5%	3	27.3%	3	**27.3%**
	Lumbosacral junction	5	5	**100.0%**	4	**80.0%**	0	0.0%	3	**60.0%**	2	40.0%	1	**20.0%**
Total		**39**	**24**	**61.5%**	**34**	**87.2%**	**3**	**7.7%**	**22**	**56.4%**	**15**	**38.5%**	**7**	**17.9%**
Grand Total		**90**	**33**	**36.7%**	**64**	**71.1%**	**24**	**26.7%**	**37**	**41.1%**	**23**	**25.6%**	**10**	**11.1%**

Table 2.3a Prevalence of selected features of axial arthropathies at a segment level, for three species of large cat, age classes combined. Key findings are highlighted in red.

Species	Axial Segment	Total	Mild facet OA		Moderate facet OA		Severe facet OA	
			Number	Percent	Number	Percent	Number	Percent
Cheetah	T1-13	6	0	0.0%	0	0.0%	0	0.0%
	L1-7	20	2	10.0%	0	0.0%	1	5.0%
	Lumbosacral junction	1	0	0.0%	0	0.0%	0	0.0%
Total		**27**	**2**	**7.4%**	**0**	**0.0%**	**1**	**3.7%**
Tiger	C1-T1	5	0	0.0%	1	20.0%	1	20.0%
	T1-T13	13	1	7.7%	0	0.0%	0	0.0%
	Thoracolumbar junction	5	0	0.0%	0	0.0%	0	0.0%
	L1-L7	11	0	0.0%	3	27.3%	0	0.0%
	Lumbosacral junction	5	1	20.0%	0	0.0%	0	0.0%
Total		**39**	**2**	**5.1%**	**4**	**10.3%**	**1**	**2. 6%**
Grand Total		**90**	**4**	**4.4%**	**4**	**4.4%**	**2**	**2.2%**

Table 2.3b Prevalence of severity grades for facet joint osteoarthritis at an axial segment level, for the cheetah and tiger, age classes combined.

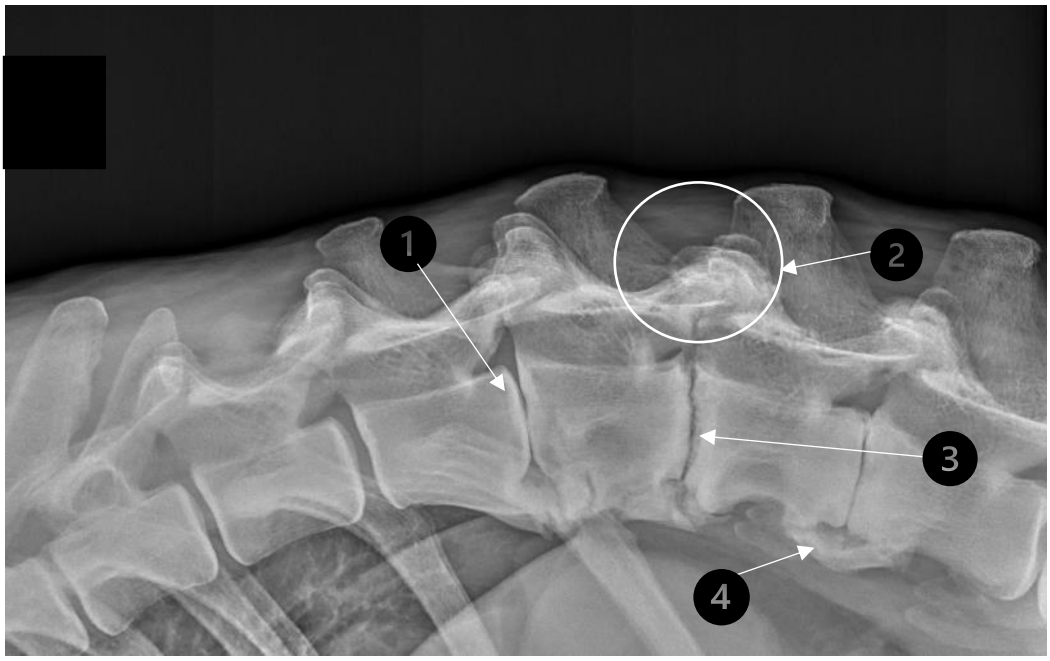

Figure 2.6 Lateral radiograph of the thoracolumbar junction and cranial lumbar spine of a geriatric (21 y) Malayan tiger showing intervertebral disc space collapse (1), facet joint osteoarthritis (2), endplate sclerosis and lucency (3), and bridging spondylosis (4).

2.3.6 Frequency and distribution of disease at the intervertebral joint level

Of the 69 arthropathy positive axial segments where more than one intervertebral joint was captured on imaging, 59.4% had disease in more than one intervertebral joint (Table 2.3c). This was most apparent in the cervical spine with 91.7% of segments (11/12) showing disease affecting more than one intervertebral joint compared with 45.8% (11/24) of thoracic and 57.6% (19/33) of lumbar segments. The highest percentage of affected joints within segment was found for the cervical spine, with the thoracic recording the lowest. The tiger recorded the highest rate of intervertebral joint disease across the vertebral column.

Axial Segment	Species	intervertebral joints captured		intervertebral joints arthropathy-positive		
		Average Number	Range	Average Number	Range	Percentage of joints arthropathy-positive
C1-T1	Cheetah	N/A	N/A	N/A	N/A	N/A
	Lion	5.571	4-7	2.29	1-4	41.03%
	Tiger	3.8	2-6	2.6	2-4	68.42%
T1-13	Cheetah	10.83	10-12	1	1	9.23%
	Lion	8.8	5-12	2	1-4	22.73%
	Tiger	8.62	3-12	2.38	1-6	27.68%
L1-7	Cheetah	5.25	1-6	1.9	1-4	36.19%
	Lion	5.17	2-6	1.83	1-4	35.48%
	Tiger	4.1	1-6	2	1-5	48.78%

Table 2.3c Frequency of intervertebral joint disease for the cervical, thoracic and lumbar segments for three species of large cat, age classes combined.

2.3.7 Unifocal versus multifocal distribution of arthropathies in the axial skeleton

There were 72 arthropathy-positive studies that had multiple axial segments imaged (Table 2.4). Seventy-one percent (51/72) of studies recorded a unifocal distribution, compared with 29.2% (21/72) for a multifocal distribution. The relative prevalence differed with both age class and species. Cheetahs demonstrated a greater propensity for unifocal disease (93.8%), compared with both lions and tigers (60% and 45% respectively). The prevalence of multifocal arthropathies increased with age, with 50% of geriatric animals showing multifocal axial arthropathy. In comparison, there were no observations for multifocal arthropathy in the young adult age group. The average number of segments imaged for a finding of a unifocal versus multifocal distribution was 3.8 and 4.4 respectively.

			Unifocal Distribution			Multifocal Distribution		
Species	Age class when radiographed	Total number	Animals with unifocal distribution		Average segments imaged	Animals with multifocal distribution		Average segments imaged
Cheetah	Young Adult	4	4	100%	4	0	0%	N/A
	Adult	11	10	90.9%	4.3	1	9.1%	4
	Senior	8	8	100%	4	0	0%	N/A
	Geriatric	9	8	88.9%	3.25	1	11.1%	5
Total		**32**	**30**	**93.8%**		**2**	**6.3%**	
Lion	Young Adult	4	4	100%	4.25	0	0%	N/A
	Adult	5	4	80%	4.25	1	20%	6
	Senior	4	1	25%	4	3	75%	4.33
	Geriatric	7	3	42.9%	3.33	4	57.1%	4.5
Total		**20**	**12**	**60%**		**8**	**40%**	
Tiger	Young Adult	2	2	100%	2.5	0	0%	N/A
	Adult	5	3	60%	4	2	40%	2.5
	Senior	5	3	60%	4	2	40%	4
	Geriatric	8	1	12.5%	4	7	87.5%	4.57
Total		**20**	**9**	**45%**		**11**	**55%**	
Grand Total		**72**	**51**	**70.8%**		**21**	**29.2%**	

Table 2.4 Unifocal versus multifocal distribution of axial arthropathies for three species across four age classes.

2.3.8 Nature of arthropathies within the axial skeleton

A total of 115 arthropathy positive axial segments from 59 animals were assessed for the nature of axial arthropathies. Fourteen animals occurred in more than one age class**,** and 22 contributed more than one axial segment within age class (Appendix 2.9 a-b). Of the 115 segments assessed, 94.8% (109/115) were classified as representing a degenerative arthropathy. In 97.3% (106/109) of degenerative axial segments, no inciting cause for degeneration could be identified. Included were 23 segments from 18 animals displaying radiographic lucency of one or more endplates. There were three cases where a causative factor was established. A history of trauma leading to spinal DJD was noted for both a lion with severe multifocal cervical spinal disease and a lioness with sacroiliac joint fusion due to a pelvic fracture incorporating the sacroiliac joint. The third case was degeneration of the thoracolumbar junction in an adult tiger with a suspected congenital or developmental abnormality at this site.

Six axial segments from five animals were determined to represent nondegenerative arthropathies. Of these, three segments from two animals involved traumatic fractures, two segments demonstrated spondylolisthesis, and one case represented a possible congenital or developmental disease of unilateral narrowing of the lumbosacral junction, with no cause identified in the medical records. A transition to a degenerative arthropathy could only be determined for one of these six segments, where a traumatic fracture of the sacrum extending into the adjacent sacroiliac joint resulted in complete fusion of the joint unilaterally (see previously). This transition was detected on reimaging several years (4.8 years) after the traumatic episode.

2.4 Discussion

This study has provided evidence that axial arthropathies in captive lions, tigers and cheetahs are common, and that the overwhelming majority of lesions are degenerative in nature. Whilst the prevalence of axial arthropathies was similar between the three species, there were few other commonalities, with each species demonstrating its own unique pattern of disease.

2.4.1 Commonalities

Age was the defining predictor for the detection of axial arthropathies. All species demonstrated increased prevalence with advancing age, confirming previous observations.[1; 3; 22] However, the effect of increasing age was site dependent. Although arthropathy prevalence increased with age in the cervical, thoracic and lumbar spines, this was not replicated in the thoracolumbar, and lumbosacral junctions, nor the sacroiliac joints. This has not been reported previously. The other similarities between the three species involved the prevalence of lumbar DJD, with a predisposition for multiple intervertebral joint involvement, and critically, a gender effect, with males of all species significantly more likely to have disease in this location than females.

2.4.2 Patterns and characteristics of axial arthropathies in the lion, tiger and cheetah

There were many similarities, but equally critical differences, between radiographic axial arthropathies of the lion versus the tiger. The tiger was notable for recording not only the highest prevalence of disease, but also the most severe disease, exhibiting the highest prevalence for all features other than intervertebral disc mineralisation. Both species were predisposed to multifocal and widespread disease, with multiple intervertebral joint involvement detected at all levels of the vertebral column. However, the distribution pattern of disease differed according to age, and the location of arthropathies in the younger age classes differed between two species. The lion was unique amongst the large cats for a high prevalence of sacroiliac disease, which was the predominant arthropathy in this species for age classes spanning young adult to senior. In contrast, the sacroiliac joint was relatively spared in tigers, with DJD of the thoracic spine typifying arthropathies of younger tigers. However, axial DJD was remarkably similar for geriatric

lions and tigers. Both species showed a high prevalence of cervical DJD, with a similarly severe radiographic appearance including bridging spondylosis, irregular, sclerotic endplate margins with punctate points of lucency, and narrowed or obliterated intervertebral disc spaces. This cervical disease was invariably accompanied by thoracic involvement. Due to the low number of female lions in senior and geriatric age classes, this study was unable to demonstrate a male predisposition for disease of the cervical spine in this species, as has been previously reported.[23] As such, additional studies with larger subject numbers are needed to clarify any gender effect on cervical arthropathies in this species. However, a gender effect was detected for sacroiliac joint disease, with female lions significantly more likely to show degenerative changes at this site.

In contrast to the lion and tiger, the cheetah showed a clearly differing pattern of radiographic axial arthropathies. Although all species showed lumbar disease at a similar prevalence, in the cheetah, disease was almost universally confined to this segment. Lumbar disease was evident in all age classes, and although prevalence increased with age, it is noteworthy that 23% of all adult cheetahs showed radiographic changes in their lumbar spines. Additionally, cheetah disease was significantly less severe than that seen in the larger species, with the appearance of intervertebral joint disease differing markedly between vertebral column segments. Lumbar disease was typically mild and characterised by in situ disc mineralisation, demonstrated generally as small, focal and discrete mineralisations affecting either a singular, or multiple, disc(s) within the segment. This disc mineralisation was a feature of cheetah lumbar intervertebral disease, with 75% of all arthropathy-positive lumbar segments showing this feature. Furthermore, the majority of lumbar disc mineralisations were not associated with other radiographic features of degenerative disc disease. As with the lion and tiger, disease affected multiple intervertebral joints within segment, however different to the lion and tiger, this was confined to the lumbar spine in the cheetah. Although less frequent, when thoracic disease was identified, the radiographic appearance differed, with narrowing or collapse of the intervertebral disc space seen in all cases, but no evidence of intervertebral disc mineralisation. As opposed to the lion and tiger, bridging spondylosis was only rarely detected in the cheetah. Other features infrequently identified were endplate changes of the thoracic and lumbar segments, and osteophytosis of the facet joint (facet OA).

A pattern is therefore evident, of the larger cats exhibiting more widespread and severe disease as they age, compared with the smaller unit that is the cheetah. The precise reasons for the differing distribution and character of lesions in lions, tigers and cheetahs are unknown. However research has suggested that size may be an issue, with heavy weight proposed as a contributing factor to axial DJD in larger nondomestic felid species.[9] Osteopathological studies have shown not only similarities of axial skeletal disease between the lion and tiger, but also noted that larger and heavier zoo mammals, such as bears and great apes, also demonstrate a high prevalence of spondyloarthroses.[24] The propensity for the lion and tiger to show widespread disease with severe changes to the cervical spine may be a reflection of musculoskeletal adaptations for ecological requirements. The 'stalk and ambush' hunting style of these larger species demand adaptations for power and strength over speed, and consequently they are characterised by larger and heavier skulls, shorter heavily muscled cervical spines, and shorter more muscular limbs. In contrast, the cheetah is a specialist cursorial predator, designed for speed characterised by rapid acceleration over short distances. A relatively small head, streamlined body and elongated distal limbs are combined with an increased length of lumbar spine, and a vertebral column capable of hyperextension and flexion.[12; 13; 25] Thus, the cheetah presents a vastly different musculoskeletal and biomechanical picture. However, how these ecologically driven anatomical and physicomechanical differences contribute to differences in vertebral stresses and therefore spinal disease is currently unclear.

2.4.3 The association between enclosure size and axial arthropathies in cheetahs

One of the most significant findings of this study was that cheetahs held in urban zoos not only had a higher prevalence, but also more severe axial disease, than those held in open-range facilities. Additionally, less disease, and less severe disease, was found in animals from Institution A. Explanations for these findings can only be speculative, as the effects of larger enclosures on the activities of nondomestic felids are unknown. However, it is reasonable to consider that larger enclosures may allow the expression of more naturalistic behaviours and gaits. Reduced activity levels in captive-held large cats compared to their free-ranging counterparts has been suggested[26] and proposed as a possible explanation for degenerative musculoskeletal conditions in captivity.[2] Supporting evidence has been provided by a recent study of captive tigers given

access to vertical feeding poles, where it was suggested that the subsequent higher activity levels suppressed the development of spondyloarthroses.[27] Tigers differ anatomically, biomechanically and ecologically to the cheetah, thus whether it is appropriate to extrapolate these findings to those of this study is unclear, however the comparison does raise the question as to whether this too might be the case for cheetahs. In addition, human studies have demonstrated a link between activity levels and lumbar disc disease, with symptomatic disease less common amongst athletes than control subjects, and an association found between a sedentary lifestyle and degenerative lumbar effects.[28; 29]

2.4.4 The features of axial arthropathies of lions, tigers and cheetahs

This study is the first to report the relative prevalence of intervertebral disc mineralisation for the lion, tiger, and cheetah. A species difference was detected. Cheetahs demonstrated a high prevalence of disc mineralisation, confined to the lumbar spine, that typically involved more than one disc within the segment. Disc mineralisation in this species was rarely associated with other radiographic degenerative features of axial arthropathy. In contrast to the cheetah, although a smaller percentage of lions showed intervertebral disc mineralisation, for those cases where it was detected, disc mineralisation was consistently evident across multiple axial segments. However, as with the cheetah, disc mineralisation in the lion was rarely associated with other radiographic features of intervertebral joint degeneration. Similar to lions, intervertebral disc mineralisation was an infrequent finding in tigers. However, all cases were confined to the lumbar spine and were associated with other radiographic features of intervertebral joint degeneration. Thus, intervertebral disc mineralisation in the tiger was associated with more severe disease than that seen in the other two species.

This study is also the first to report the radiographic detection of facet OA in large cats. Four tigers and three cheetahs demonstrated this feature, with a notable absence in lions. Distribution differed, with facet OA confined to the lumbar spine in the cheetah, compared with a more widespread distribution for the tiger. For both species, facet OA was invariably associated with other radiographic features of intervertebral joint degeneration. Although the total number of individual animals with this feature was low, the finding of radiographic facet OA in large cats is

significant. Facet OA is considered difficult to demonstrate on plain radiography in companion animals,[14] suggesting that the findings from this study may be an under-representation of true prevalence. The prevalence and significance of radiographic facet OA differs according to species. Rarely reported as a radiographic finding in the domestic cat,[30] facet OA in the dog is often considered an incidental finding.[31] In contrast, in human medicine facet OA is generally considered significant, occurring either as part of broader degenerative disc disease, or occurring independently. Regardless, due to the identified rich innervation of the facet joint in humans, degenerative changes are often considered clinically significant, associated with pain and dysfunction.[32-34] Whether this may also be the case for large cats requires further investigation.

In addition to finding osteophytosis of the facet joint, osteophytosis of the sacroiliac joint was also detected. This feature was seen predominantly in lions, with only two additional cases detected in tigers. This study is the first to identify this radiographic change in these two species, with a review of the literature failing to identify any reference to sacroiliac OA in either large cats, domestic cats or dogs. However, degeneration of the sacroiliac joint is commonly recognised in human medicine,[35] with both sclerosis and osteophytosis identified on radiography. These degenerative changes are considered chronic in nature and most likely a reflection of repeated microtrauma at the joint.[36] The clinical significance of sacroiliac osteoarthritis in human medicine is currently unclear, with studies reporting both an increased incidence of radiographic OA in patients with sacroiliac joint pain[37] and conversely a high incidence of asymptomatic patients with evidence of sacroiliac joint disease on imaging.[35] Whether these inconsistencies are also seen in large cats is yet to be determined, as the clinical significance of sacroiliac OA in large cats is currently unknown.

As with many features of axial arthropathies, a definite species difference was evident for bridging spondylosis deformans. Whilst rare in the cheetah, bridging spondylosis was evident in the larger Panthera species. The tiger was overrepresented, recording both the highest prevalence of bridged joints within segment, and the most widespread distribution, with detection at all levels of the vertebral column. In all three species, bridging spondylosis deformans was considered a significant finding, with 80% of all bridged intervertebral joints

showing associated degenerative changes. Although the association between bridging spondylosis and intervertebral disc degeneration has been noted previously in large cats[3] and companion animals,[30; 31; 38] no causal effect has yet been established. However, a 'domino effect' has been recognised. Bridging of multiple contiguous vertebrae results in altered biomechanics and increased local stresses, with subsequent dynamic overload and degeneration of adjacent intervertebral joints. In particular, the overloaded disc immediately cranial to the fused spinal segment is considered to be most vulnerable.[39-42] Over 50 % of potential 'domino effect' sites identified in this study showed degeneration of the intervertebral joint immediately cranial to the area of ankylosis. This finding lends further weight to the significance of bridging spondylosis in large cats, particularly when involving multiple contiguous vertebrae.

Diffuse idiopathic skeletal hyperostosis (DISH) of the axial skeleton has previously been reported in tigers,[27] but was not seen in the current study. DISH is defined as a systemic disorder, resulting in ossification of soft tissues including the ventral longitudinal ligament of the spine, as well as entheses generally. As such, DISH can resemble the changes seen with spondylosis deformans, and the two can be difficult to distinguish radiographically.[43] Criteria for a radiographic diagnosis of DISH differ between authors.[31; 43; 44] This study adopted the conservative and widely accepted Resnick criteria for the diagnosis of DISH in humans.[45] These included the "presence of flowing calcification and ossification along the ventrolateral aspect of at least three to four contiguous vertebral bodies", and "an absence of degenerative disease of the associated intervertebral joint(s)". On this basis alone, DISH was ruled out for all cases of bridging spondylosis involving three or more contiguous vertebrae, as without exception, all bridged regions showed other degenerative radiographic features.

This study is the first to report radiographic endplate lucency in the lion, tiger, and cheetah. As this feature is most often associated with severe disease of the intervertebral joint, this is considered a finding of particular significance. Radiographic endplate lucency was demonstrated by 16 animals, comprising 9 tigers, 4 lions and 3 cheetahs. Without exception, endplate lucency was detected as part of a constellation of radiographic features that included intervertebral space narrowing or collapse, endplate sclerosis and spondylosis deformans.

Endplate lucency was most commonly seen in the tiger, with a high level of detection in the cervical spine, but all levels of the vertebral column were affected. Of particular significance was the observation that endplate lucency was detected at multiple noncontiguous sites across the vertebral column for two-thirds of all tiger cases. This result is possibly an underestimation of the true frequency, as it was unusual for an individual animal to undergo a complete axial radiographic survey. Similar to the tiger, the lion also showed a high level of detection in the cervical spine, in addition to the thoracic spine and lumbosacral junction, whereas lucency was confined to the lumbar and thoracic spine of the cheetah.

There are multiple possible aetiologies for radiographic endplate lucency. Although potentially associated with benign pathologies, the endplate changes detected in this study did not resemble these diseases. In contrast, the association with sclerosis was a key indicator that endplate lucency was associated with more severe, degenerative and destructive disease. Discospondylitis is the most commonly reported cause of endplate lucency in companion animal medicine. Defined as inflammation and infection of an intervertebral disc and its adjacent vertebral endplates, the end result, particularly if untreated, is a degradative effect on both structures.[30; 31; 42; 46] Precedence in the literature for discospondylitis in captive large cats is confined to an isolated case in a leopard.[47] Discospondylitis is uncommon in the domestic cat[48] but is not infrequently diagnosed in large breed dogs.[30] The radiographic appearance of canine discospondylitis has been well described, with advanced discospondylitic lesions resembling radiographic features of chronic intervertebral disc disease, or conversely, if resolved, spondylosis deformans.[49] Thus discospondylitis and chronic intervertebral disc disease can be difficult to differentiate radiographically.

The intervertebral joint changes identified in this study are consistent with some radiographic features of later stage or chronic unresolved discospondylitis, with endplate lucency potentially denoting lysis or bone destruction, and the associated sclerosis and bridging implying chronicity. Alternative causes of endplate lucency, associated with other degenerative or destructive disease, are also recognised. Irregularities of the endplate surface due to sclerotic bone can create relative radiographic endplate lucency that is difficult to differentiate from true lucency.

True lucency of the endplate also occurs with Schmorl's nodes, a protrusion of disc material through the endplate into the vertebral body.[50] The prevalence and significance of Schmorl's nodes in large cats is yet to be established, however their frequency and clinical relevance in companion animal medicine is uncertain, with reports confined to a few cases in the canine literature.[51-53]

A recent study of human cadaveric lumbar vertebral endplates found that Schmorl's nodes were but one of several pathologies resulting in endplate defects. Erosive lesions were also identified.[50] As radiographic examination was not included in the cadaveric study, the radiographic appearance of these lesions cannot be commented on. However, the authors did note the relative insensitivity of plain radiography for the detection of endplate disease, and the tendency to attribute changes to Schmorl's nodes, when in fact erosive lesions occurred at an almost equivalent frequency. These findings have relevant implications for our observations of radiographic endplate lucency in large cats.

Thus several possible pathologies have been identified to explain this study's findings of endplate lucency in large cats. It is the conclusion of this study that lucency in these species represents either chronic unresolved discospondylitis, or noninfectious intervertebral joint degeneration, including an equivalent of the erosive lesions found in the endplates of human lumbar discs. However, additional diagnostic tests are needed for further differentiation and clarification. Of note, the failure to identify any matched clinical record entries regarding discospondylitis does raise the prospect as to whether large cats suffer from atypical or clinically silent discospondylitis. Alternatively, our observations may represent the first report of endplate lucency as a result of degenerative disc disease in these species.

2.4.5 Recommended protocol for the radiographic detection of axial DJD

This comprehensive study, investigating multiple indices and aspects of axial arthropathies, has shown that not only were they common, and mostly degenerative, in captive lions, tigers and cheetahs, but that critically, different species showed distinct patterns of disease. As a result of the scope of this collective information, the following recommendations for maximising radiographic detection of axial DJD are offered, particularly in regard to the prioritisation of

imaging when survey radiography of the entire spine is not feasible. The thoracic spine of older tigers, and the sacroiliac joints of lions of all ages, should be assessed. For both species, a propensity for severe cervical pathology in geriatric animals mandates imaging of this level in the older age classes. In addition, due to both the high incidence of multiple intervertebral joint involvement, and the tendency for widespread or multifocal pathology, comprehensive imaging of the vertebral column is indicated in susceptible individuals. In comparison, for the cheetah, particular attention should be directed at the lumbar spine. Due to the relatively young age at detection, lumbar imaging is recommended for cheetahs older than four years.

For all images of the vertebral column, although orthogonal projections are preferred, lateral views generally suffice, with the exception of the sacroiliac joint, which can be assessed from a standard ventrodorsal projection of the pelvis. For older lions and tigers in particular, distraction of the forelimbs caudad, to assist radiographic interpretation of caudal cervical and cranial thoracic segments, is warranted, and may prove rewarding. The findings from this study indicate that this approach is necessary in order to fully appreciate the extent of axial DJD impacting these species.

2.5 Conclusion

This radiological investigation of axial DJD in captive lions, tigers and cheetahs is the largest study of its kind undertaken to date. In addition, this is the first study to report on axial arthropathies in the cheetah. The findings provide invaluable information to both zoo veterinarians and managers concerning key decisions related to axial DJD, including when to commence axial radiography as part of routine health assessment, individuals most at risk, and where best to prioritise imaging. This is critical, as spinal radiographs in all large cats require general anaesthesia, and the prolonged time required for complete radiological survey of the axial skeleton creates additional risks to the patient.

This study also indicates that cheetahs in open-range zoos may have a significantly lower prevalence of axial arthropathies than those housed in smaller enclosures. This animal welfare issue should be considered when cheetah enclosures are being designed, and when managers are considering whether to include cheetahs in a zoo collection. Based on these findings for the cheetah, additional studies examining the impact of enclosure design on the prevalence of axial arthropathies in other captive-held large cat species are warranted. This study is also the first to report the presence of facet and sacroiliac joint OA, and endplate lucency, in large cats. These lesions potentially have significant clinical implications and merit additional study.

Lastly, arthropathies of the axial skeleton do not occur in isolation, with disease also occurring in multiple joints in the appendicular skeleton. Therefore, additional studies are needed to determine the nature, distribution, and severity of arthropathies in the appendicular skeleton of captive large cats, and how these relate to lesions of the axial skeleton.

Appendix 2.1 Frequency distribution of predictors against arthropathy status for the axial skeleton.

			Arthropathy Negative		**Arthropathy Positive**	
Predictor	**Sub-category**	**Total Count**	Number	Percent	Number	Percent
Species	Cheetah	173	140	80.9%	33	19.1%
	Lion	59	38	64.4%	21	35.6%
	Tiger	81	60	74.1%	21	25.9%
Gender	Female	141	109	77.3%	32	22.7%
	Male	172	129	75.0%	43	25.0%
Age Class	Young Adult	122	112	91.8%	10	8.2%
	Adult	87	68	78.2%	19	21.8%
	Senior	60	40	66.7%	20	33.3%
	Geriatric	44	18	40.9%	26	59.1%
Grand Total		**313**	**238**	**76.0%**	**75**	**24.0%**
Cheetah Enclosure Size						
Urban vs Open Range	Open Range	119	102	85.7%	17	14.3%
	Urban	54	38	70.4%	16	29.6%
Institution A vs Other	Institution A	85	77	90.6%	8	9.4%
	Other	88	63	71.6%	25	28.4%
Grand Total		**173**	**140**	**80.9%**	**33**	**19.1%**

Appendix 2.2 P values from modelling of predictors against arthropathy status for the axial skeleton. Significant associations are highlighted in red, trending associations in purple.

Predictor	**P value**
Age	**<0.001**
Species	**0.088**
Gender	0.547
Age Species Interaction	0.719
Species Gender Interaction	0.267
Age Gender Interaction	0.715

Cheetah Enclosure Size	Urban vs Open Range	Institution A vs Other
Enclosure Size	**0.067**	**0.008**
Enclosure Size Age Interaction	0.587	0.806
Enclosure Size Gender Interaction	0.479	0.11

Appendix 2.3 Predicted (non back transformed) means and standard errors of differences, used for manual calculation of pairwise differences for significant predictors of arthropathies for the axial skeleton.

Age Class	**Predicted Means**
Young Adult	-2.467
Adult	-1.258
Senior	-0.859
Geriatric	0.365
Standard errors of differences	
Average:	0.448
Maximum:	0.4907
Minimum:	0.4062

Appendix 2.4 a-f Frequency distribution of predictors against arthropathy status at an axial segment level.

Appendix 2.4a Axial Segment level; C1-T1.

Axial Segment level - C1-T1

Predictor	Sub-category	Total Count	Arthropathy Negative		Arthropathy Positive	
			Number	Percent	Number	Percent
Species	Cheetah	38	38	100.0%	0	0.0%
	Lion	29	23	79.3%	6	20.7%
	Tiger	19	14	73.7%	5	26.3%
Gender	Female	38	35	92.1%	3	7.9%
	Male	48	40	83.3%	8	16.7%
Age Class	Young Adult	41	41	100.0%		0.0%
	Adult	23	21	91.3%	2	8.7%
	Senior	13	10	76.9%	3	23.1%
	Geriatric	9	3	33.3%	6	66.7%
Grand Total		**86**	**75**	**87.2%**	**11**	**12.8%**

Appendix 2.4b Axial Segment level; T1-T13.

Axial Segment level T1-T13

Predictor	Sub-category	Total Count	Arthropathy Negative		Arthropathy Positive	
			Number	Percent	Number	Percent
Species	Cheetah	131	124	94.7%	7	5.3%
	Lion	31	26	83.9%	5	16.1%
	Tiger	47	33	70.2%	14	29.8%
Gender	Female	99	88	88.9%	11	11.1%
	Male	110	95	86.4%	15	13.6%
Age Class	Young Adult	78	78	100.0%		0.0%
	Adult	62	57	91.9%	5	8.1%
	Senior	37	28	75.7%	9	24.3%
	Geriatric	32	20	62.5%	12	37.5%
Grand Total		**209**	**183**	**87.6%**	**26**	**12.4%**
		Cheetah Enclosure Size				
Urban vs Open Range	Open Range	85	81	95.3%	4	4.7%
	Urban	46	43	93.5%	3	6.5%
Institution A vs Other	Institution A	68	66	97.1%	2	2.9%
	Other	63	58	92.1%	5	7.9%
Grand Total		**131**	**124**	**94.7%**	**7**	**5.3%**

Appendix 2.4c Axial Segment level; Thoracolumbar junction.

Axial Segment level - Thoracolumbar junction						
Predictor	**Sub-category**	**Total Count**	**Arthropathy Negative**		**Arthropathy Positive**	
			Number	**Percent**	**Number**	**Percent**
Species	Cheetah	118	118	100.0%	0	0.0%
	Lion	34	33	97.1%	1	2.9%
	Tiger	43	38	88.4%	5	11.6%
Gender	Female	101	100	99.0%	1	1.0%
	Male	94	89	94.7%	5	5.3%
Age Class	Young Adult	76	76	100.0%	0	0.0%
	Adult	60	59	98.3%	1	1.7%
	Senior	29	28	96.6%	1	3.4%
	Geriatric	30	26	86.7%	4	13.3%
Grand Total		**195**	**189**	**96.9%**	**6**	**3.1%**

Appendix 2.4d Axial Segment level; L1-L7.

Axial Segment level L1-L7						
Predictor	**Sub-category**	**Total Count**	**Arthropathy Negative**		**Arthropathy Positive**	
			Number	**Percent**	**Number**	**Percent**
Species	Cheetah	152	125	82.2%	27	17.8%
	Lion	45	38	84.4%	7	15.6%
	Tiger	65	54	83.1%	11	16.9%
Gender	Female	125	110	88.0%	15	12.0%
	Male	137	107	78.1%	30	21.9%
Age Class	Young Adult	97	92	94.8%	5	5.2%
	Adult	77	65	84.4%	12	15.6%
	Senior	47	37	78.7%	10	21.3%
	Geriatric	41	23	56.1%	18	43.9%
Grand Total		**262**	**217**	**82.8%**	**45**	**17.2%**
Cheetah Enclosure Size						
Urban vs Open Range	Open Range	101	87	86.1%	14	13.9%
	Urban	51	38	74.5%	13	25.5%
Institution A vs Other	Institution A	78	72	92.3%	6	7.7%
	Other	74	53	71.6%	21	28.4%
Grand Total		**152**	**125**	**82.2%**	**27**	**17.8%**

Appendix 2.4e Axial segment level; Lumbosacral junction.

Axial Segment level Lumbosacral junction						
Predictor	**Sub-category**	**Total Count**	**Arthropathy Negative**		**Arthropathy Positive**	
			Number	**Percent**	**Number**	**Percent**
Species	Cheetah	115	114	99.1%	1	0.9%
	Lion	39	33	84.6%	6	15.4%
	Tiger	65	60	92.3%	5	7.7%
Gender	Female	101	95	94.1%	6	5.9%
	Male	118	112	94.9%	6	5.1%
Age Class	Young Adult	88	88	100.0%		0.0%
	Adult	60	57	95.0%	3	5.0%
	Senior	40	38	95.0%	2	5.0%
	Geriatric	31	24	77.4%	7	22.6%
Grand Total		**219**	**207**	**94.5%**	**12**	**5.5%**

Appendix 2.4f Axial Segment level; Sacroiliac joint.

Axial Segment level Sacroiliac joint						
Predictor	**Sub-category**	**Total Count**	**Arthropathy Negative**		**Arthropathy Positive**	
			Number	**Percent**	**Number**	**Percent**
Species	Cheetah	75	75	100.0%	0	0.0%
	Lion	26	14	53.8%	12	46.2%
	Tiger	41	39	95.1%	2	4.9%
Gender	Female	68	57	83.8%	11	16.2%
	Male	74	71	95.9%	3	4.1%
Age Class	Young Adult	64	59	92.2%	5	7.8%
	Adult	33	30	90.9%	3	9.1%
	Senior	26	22	84.6%	4	15.4%
	Geriatric	19	17	89.5%	2	10.5%
Grand Total		**142**	**128**	**90.1%**	**14**	**9.9%**

Appendix 2.5 P values for the modelling of associations between predictors and arthropathies at six axial segment levels. Significant associations are highlighted in red, trending associations in purple.

							Enclosure Size	
Axial Segment	**Age Class**	**Species**	**Gender**	**Age Species Inter-action**	**Age Gender Inter-action**	**Gender Species Inter-action**	**Urban vs Open Range**	**Institution A vs Other**
C1-T1	0.024	0.803	0.569	1	0.997	0.751	N/A[1]	N/A
T1-13	0.011	<0.001	0.766	0.797	0.516	0.397	0.66	0.222
Thoracolumbar junction	0.2	0.372	0.118	1	0.999	0.994	N/A	N/A
L1-7	<0.001	0.899	0.024	0.255	0.822	0.763	0.08	0.002
Lumbosacral junction	0.112	0.016	0.974	0.905	0.991	0.904	N/A	N/A
Sacroiliac joint	0.755	0.003	0.023	1	0.917	0.5	N/A	N/A

[1] N/A – Non Applicable no cheetah disease detected

Appendix 2.6 a-e Significant predictors for arthropathy at an axial segment level. Predicted mean prevalence, standard error of the mean, and least significant differences of predictions (5%) level are presented.

Appendix 2.6a C1-T1: significant association with age class.

Age Class	Prediction[2]	s.e.[3]	Least significant differences of predictions (5% level)				
Young Adult	0	0.00048	Young Adult	*			
Adult	0.087	0.05875	Adult	0.1169	*		
Senior	0.2308	0.11685	Senior	0.2325	0.2602	*	
Geriatric	0.6667	0.15713	Geriatric	0.3126	0.3337	0.3896	*
				Young Adult	Adult	Senior	Geriatric

Appendix 2.6b T1-13: significant association with species and age class.

Species	Prediction	s.e.	Least significant differences of predictions (5% level)			
Cheetah	0.0534	0.0196	Cheetah	*		
Lion	0.1613	0.06602	Lion	0.1358	*	
Tiger	0.2979	0.0665	Tiger	0.1367	0.1847	*
				Cheetah	Lion	Tiger

Age Class	Prediction	s.e.	Least significant differences of predictions (5% level)				
Young Adult	0	0.00035	Young Adult	*			
Adult	0.0806	0.03458	Adult	0.0682	*		
Senior	0.2432	0.07053	Senior	0.1391	0.1549	*	
Geriatric	0.375	0.08558	Geriatric	0.1687	0.182	0.2187	*
				Young Adult	Adult	Senior	Geriatric

[2] prediction denotes average predicted prevalence as a %

[3] s.e. denotes standard error of the mean

Appendix 2.6c L1-L7: significant association with age, gender and cheetah enclosure size.

Age Class	Prediction	s.e.	Least significant differences of predictions (5% level)				
Young Adult	0.0515	0.02238	Young Adult	*			
Adult	0.1558	0.04131	Adult	0.0925	*		
Senior	0.2128	0.05956	Senior	0.1253	0.1427	*	
Geriatric	0.425	0.07816	Geriatric	0.1601	0.1741	0.1935	*
				Young Adult	Adult	Senior	Geriatric

Gender	Prediction	s.e.
Female	0.1129	0.02842
Male	0.219	0.03525

LSDs not calculated due to binomial variable

Enclosure Size	Prediction	s.e.
Institution A	0.0769	0.03017
Other	0.2838	0.05224

LSDs not calculated due to binomial variable

Appendix 2.6d Lumbosacral junction: significant association with species.

Species	Prediction	s.e.	Least significant differences of predictions (5% level)			
Cheetah	0.0087	0.00862	Cheetah	*		
Lion	0.15385	0.05777	Lion	0.1151	*	
Tiger	0.0625	0.03026	Tiger	0.062	0.1285	*
				Cheetah	Lion	Tiger

Appendix 2.6e Sacroiliac joint: significant association with species and gender

Species	Prediction	s.e.	Least significant differences of predictions (5% level)			
Cheetah	0	0.00022	Cheetah	*		
Lion	0.4615	0.09777	Lion	0.1933	*	
Tiger	0.0488	0.03364	Tiger	0.0665	0.2044	*
				Cheetah	Lion	Tiger

Gender	Prediction	s.e.
Female	0.1618	0.04463
Male	0.0405	0.02269

LSDs not calculated due to binomial variable

Appendix 2.7 a-c Frequency of axial segment arthropathies for cheetah, lion and tiger, at the level of 'animal within an age class'. Key findings are highlighted in red.

Appendix 2.7a Cheetah

		CHEETAH		
Age Class	**Axial Segment**	**Total Number**	**Number arthropathy positive**	**Percent arthropathy positive**
Young Adult	C1-T1	19	0	0.0%
	T1-13	54	0	0.0%
	Thoracolumbar junction	48	0	0.0%
	L1-7	62	4	6.5%
	Lumbosacral junction	54	0	0.0%
	Sacroiliac joint	34	0	0.0%
Young Adult Total		**271**	**4**	**1.5%**
Adult	C1-T1	12	0	0.0%
	T1-13	36	2	5.6%
	Thoracolumbar junction	36	0	0.0%
	L1-7	42	10	23.8%
	Lumbosacral junction	28	0	0.0%
	Sacroiliac joint	16	0	0.0%
Adult Total		**170**	**12**	**7.1%**
Senior	C1-T1	5	0	0.0%
	T1-13	22	3	13.6%
	Thoracolumbar junction	19	0	0.0%
	L1-7	27	6	22.2%
	Lumbosacral junction	19	0	0.0%
	Sacroiliac joint	15	0	0.0%

Senior Total		**107**	**9**	**8.4%**
Geriatric	C1-T1	2	0	0.0%
	T1-13	19	2	10.5%
	Thoracolumbar junction	15	0	0.0%
	L1-7	21	7	33.3%
	Lumbosacral junction	14	1	7.1%
	Sacroiliac joint	10	0	0.0%
Geriatric Total		**81**	**10**	**12.3%**
Grand Total		**629**	**35**	**5.6%**

Appendix 2.7b Lion

Age Class	Axial Segment	LION		
		Total Number	Number arthropathy positive	Percent arthropathy positive
Young Adult	C1-T1	16	0	0.0%
	T1-13	13	0	0.0%
	Thoracolumbar junction	17	0	0.0%
	L1-7	16	0	0.0%
	Lumbosacral junction	14	0	0.0%
	Sacroiliac joint	11	4	36.4%
Young Adult Total		**87**	**4**	**4.6%**
Adult	C1-T1	5	1	20.0%
	T1-13	8	0	0.0%
	Thoracolumbar junction	7	0	0.0%
	L1-7	12	0	0.0%
	Lumbosacral junction	9	2	22.2%
	Sacroiliac joint	8	3	37.5%
Adult Total		**49**	**6**	**12.2%**
Senior	C1-T1	4	2	50.0%
	T1-13	6	2	33.3%
	Thoracolumbar junction	3	1	33.3%
	L1-7	7	3	42.9%
	Lumbosacral junction	8	2	25.0%
	Sacroiliac joint	4	3	75.0%
Senior Total		**32**	**13**	**40.6%**
Geriatric	C1-T1	4	3	75.0%
	T1-13	4	3	75.0%

	Thoracolumbar junction	7		0.0%
	L1-7	10	4	40.0%
	Lumbosacral junction	8	2	25.0%
	Sacroiliac joint	3	2	66.7%
Geriatric Total		**36**	**14**	**38.9%**
Grand Total		**204**	**37**	**18.1%**

Appendix 2.7c Tiger

Age Class	Axial Segment	TIGER		
		Total Number	Number arthropathy positive	Percent arthropathy positive
Young Adult	C1-T1	6	0	0.0%
	T1-13	11	0	0.0%
	Thoracolumbar junction	11	0	0.0%
	L1-7	19	1	5.3%
	Lumbosacral junction	20	0	0.0%
	Sacroiliac joint	19	1	5.3%
Young Adult Total		**86**	**2**	**2.3%**
Adult	C1-T1	6	1	16.7%
	T1-13	18	3	16.7%
	Thoracolumbar junction	17	1	5.9%
	L1-7	23	2	8.7%
	Lumbosacral junction	23	1	4.3%
	Sacroiliac joint	9	0	0.0%
Adult Total		**96**	**8**	**8.3%**
Senior	C1-T1	4	1	25.0%
	T1-13	9	4	44.4%
	Thoracolumbar junction	7	0	0.0%
	L1-7	13	1	7.7%
	Lumbosacral junction	13	0	0.0%
	Sacroiliac joint	7	1	14.3%
Senior Total		**53**	**7**	**13.2%**

Geriatric	C1-T1	3	3	100.0%
	T1-13	9	7	77.8%
	Thoracolumbar junction	8	4	50.0%
	L1-7	10	7	70.0%
	Lumbosacral junction	9	4	44.4%
	Sacroiliac joint	6	0	0.0%
Geriatric Total		**45**	**25**	**55.6%**
Grand Total		**280**	**42**	**15.0%**

Appendix 2.8 a –c Tabulations of average maximum severity at an axial segment level for three species, age classes pooled, arthropathy-positive segments only.

Appendix 2.8a Cheetah

CHEETAH

Segment	Total Number	average maximum severity	average maximum age class
C1-T1	0	0	N/A
T1-13	6	1.67	3
Thoracolumbar junction	0	0	N/A
L1-7	20	1.35	2.85
Lumbosacral junction	1	2	4
Sacroiliac joint	0	0	N/A

Appendix 2.8b Lion

LION

Segment	Total Number	average maximum severity	average maximum age class
C1-T1	6	2.5	3.33
T1-13	5	1.8	3.6
Thoracolumbar junction	1	1	3
L1-7	6	1.67	3.67
Lumbosacral junction	6	2	3
Sacroiliac joint	9	1.44	2.33

Appendix 2.8c Tiger

TIGER			
Segment	**Total Number**	**average maximum severity**	**average maximum age class**
C1-T1	5	2.8	3.4
T1-13	13	2	3.39
Thoracolumbar junction	5	2.4	3.6
L1-7	11	2	3.27
Lumbosacral junction	5	2.4	3.6
Sacroiliac joint	2	1.5	2

Appendix 2.8d Tabulation for the cheetah with respect to enclosure size: average maximum severity, arthropathy positive segments only, all axial segments combined, age classes pooled, urban versus open range facilities.

CHEETAH- **ALL SEGMENTS**	**Total Number**	**average maximum severity**	**average maximum age class**
Urban	8	1.89	2.89
Open Range	18	1.22	3

Appendix 2.9 a-b Nature of arthropathy: distribution of data set

Appendix 2.9a Age class within species.

Species	Age Class At Xray	Number of axial segments	% of axial segments per age class within/between species
Cheetah	Young Adult	4	11.43%
	Adult	12	34.29%
	Senior	9	25.71%
	Geriatric	10	28.57%
Cheetah Total		**35**	**30.43%**
Lion	Young Adult	4	10.53%
	Adult	5	13.16%
	Senior	13	34.21%
	Geriatric	16	42.11%
Lion Total		**38**	**33.04%**
Tiger	Young Adult	2	4.76%
	Adult	8	19.05%
	Senior	7	16.67%
	Geriatric	25	59.52%
Tiger Total		**42**	**36.52%**
Grand Total		**115**	**100.00%**

Appendix 2.9b Axial segment within species

Axial Segment	Total axial segments number	Total axial segments percent	Cheetah axial segments number	Cheetah axial segments percent	Lion axial segments number	Lion axial segments percent	Tiger axial segments number	Tiger axial segments percent
C1-T1	12	10.43%	0	0.00%	7	18.42%	5	11.90%
T1-13	26	22.61%	7	20.00%	5	13.16%	14	33.33%
Thoracolumbar junction	6	5.22%	0	0.00%	1	2.63%	5	11.90%
L1-7	45	39.13%	27	77.14%	7	18.42%	11	26.19%
Lumbosacral junction	12	10.43%	1	2.86%	6	15.79%	5	11.90%
Sacroiliac joint	14	12.17%	0	0.00%	12	31.58%	2	4.76%
Grand Total	**115**	**100.00%**	**35**	**100.00%**	**38**	**100.00%**	**42**	**100.00%**

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Chapter 3

A retrospective radiological study of arthropathies of captive lions, tigers and cheetahs

Part II: The Appendicular Skeleton

3.1 Introduction

Degenerative joint disease (DJD) is considered a major welfare concern for captive large cats,[1; 2] however little is known regarding its prevalence and distribution in these species. In the previous chapter (Chapter 2), it was shown that captive lions, tigers and cheetahs have a high prevalence of axial arthropathies which are predominantly degenerative in nature, with the three species showing very different distributions and patterns of disease. Based on these findings, the investigation of joint diseases of the appendicular skeleton for captive cheetahs, lions and tigers is equally appropriate, and particularly applicable given the paucity of information available on this topic. Reports of appendicular joint diseases of the captive lion tiger and cheetah are currently restricted to osteopathological surveys,[3-7] and a handful of case studies,[8-10] with appendicular joint disease sometimes only included as an incidental finding.[11-13]

The first objective of this study was to determine the radiographic prevalence, distribution, and features of appendicular joint disease in captive lions, tigers and cheetahs. The second objective was to determine if there was an association between radiographic arthropathy status of appendicular joints and potential causative factors for disease, including animal demographics and enclosure size (cheetah only). It was expected that the findings from this study would mirror those pertaining to the axial skeleton, with a similar prevalence of disease, the majority of lesions being degenerative in nature, and each species demonstrating its own unique pattern of disease.

3.2 Materials and Methods

3.2.1 Data acquisition and age class classification

Data acquisition is described in Chapter 2, with fourteen institutions from Australia, New Zealand and North America providing clinical records and radiographic studies of all lions, tigers and cheetahs from 1979-2019. This yielded 702 radiographic studies from 305 animals. All studies were then assessed for suitability for inclusion. Studies were included for scoring if the animal was six months or older at the time of radiographic examination with appendicular skeleton radiographs of suitable diagnostic quality. All musculoskeletal anatomy of the relevant joint was required to be included in the field of collimation for an image to be included in this study. A total of 284 radiographic studies of 149 animals, containing 1494 images of the appendicular skeleton, met the above inclusion criteria. Animals were then categorised into four age classes: young adult, adult, senior and geriatric, as described in Chapter 2.

3.2.2 Radiological scoring system design and application

A study-specific radiology scoring system, modified from protocols previously described for evaluation of the appendicular skeleton in domestic cats, was developed.[14-16] All primary joints of limbs, excluding manus and pes, were evaluated individually, with left and right joints within joint type scored independently. Appendicular joints evaluated included glenohumeral (henceforth referred to as shoulder), humero-radio-ulnar (henceforth referred to as elbow), carpal, coxofemoral, femorotibial (henceforth referred to as stifle) and tarsal joints. These joints were assessed for the presence or absence of radiographic features of appendicular arthropathy, including joint-associated mineralisation (osteophytosis, enthesophytosis, dystrophic mineralisation, osseous bodies), intracapsular soft tissue opacity, subchondral bone sclerosis and lucency, and the presence of intra-articular fracture(s). Osteophytosis and enthesophytosis were subjectively graded as mild (1), moderate (2) or severe (3). Osseous bodies were scored as either absent (0), or present (1), followed by subclassification within the categories osteochondritis dissecans (OCD), meniscal mineralisation (stifle joint only), fragment, or undefined. All other appendicular lesions were scored either as absent (0), present (1), equivocal (2), or 'unable to assess' (3).

Each captured appendicular joint then received an arthropathy score of negative or positive, with a positive score assigned if one or more of the above features were identified within the joint. The severity of the lesion was then graded as mild, moderate, or severe, based on the severity scoring systems previously described for the domestic cat.[15; 17] The extent of coverage of the femoral head within the acetabulum did not contribute to arthropathy scoring of the coxofemoral joint, unless accompanied by remodeling of the femoral head and/or acetabulum. Intrameniscal mineralisation confined to a singular, discrete focal area of mineralisation within the region of the cranial horn of the medial meniscus, with an appearance consistent with an osseous body, was identified as a meniscal ossicle,[18-22] and as such was not scored as a radiographic abnormality. The presence of a small, rounded or oval, discrete osseous body adjacent to, or articulating with, the craniolateral head of the radius, was identified as a sesamoid bone in the tendon of origin of the supinator muscle,[23-25] and was scored as such. This finding did not contribute to arthropathy scoring for the associated elbow joint. A final arthropathy score (negative, positive) was then assigned to the study, with a positive score denoting the inclusion of one or more arthropathy-positive appendicular joints.

As with the radiological study of the axial skeleton, all radiographic images were scored by the primary author (LB), with a select subset of these reviewed by a board-certified DACVR radiologist (AY). A similar validation process was undertaken as described in Chapter 2. Of the 284 studies eligible for inclusion, the initial 50 studies (17.6%) were reviewed and scored by both assessors. Subsequently, where lesions were equivocal, images were reviewed by both, and a consensus was reached. As a result, 38% (108/284) of all eligible studies were assessed by both reviewers. Both reviewers were blinded to clinical information at the time of radiological scoring. The results of all study scoring, with corresponding animal identification data, were recorded in a spreadsheet (Microsoft ® Excel 2016) database, as described in Chapter 2.

Osteopathological specimens of captive cheetahs, lions and tigers held at the School of Animal and Veterinary Sciences, University of Adelaide, were utilised as a reference for normal and pathological features. In addition, published papers describing the radiographic appearance of both normal and abnormal appendicular joints [22; 26-28] and normal anatomy [29-33] for the three

species were consulted. This information was supplemented by veterinary radiology texts for companion animals when indicated.[34; 35]

3.2.3 Determination of nature of arthropathy

Radiographic appearance of joint disease in the appendicular skeleton is not always pathognomonic for degenerative joint disease. Therefore, to assist in identifying degenerative arthropathies, matched clinical records were reviewed after radiological scoring. Based on the combination of relevant clinical entries with radiographic features detected, appendicular arthropathies were further classified into degenerative versus nondegenerative arthropathies. Subclassification within these two broad categories followed, and was based on aetiology identified from patient information, as described in Chapter 2. A developmental osteochondritis dissecans diagnosis was used in cases demonstrating both an intraarticular osseous body with an associated area of subchondral lucency.[36; 37] All arthropathies that were detected to transition from a nondegenerative to degenerative state were also identified.

3.2.4 Data preparation for analysis

Some animals had their appendicular skeleton imaged on multiple occasions and across multiple age classes. It was necessary therefore to identify and remove repeat measures prior to statistical analysis. The methodology used to determine which study to include in analysis is as described in Chapter 2. This was applied to the data sets for the appendicular skeleton in toto and the six different appendicular joint types, with the outcome that each animal could only contribute one study per age class to the data set. Due to insufficient numbers of studies, for the descriptive analyses of the most severely affected appendicular joint type, and the prevalence of features of appendicular arthropathies, age classes were pooled. In these analyses, each animal was restricted to one entry per data set, with the entry representing an aggregation of all available radiographic data for that site, for the life of the animal.

3.2.5 Statistical analysis

The radiologic scoring data was analysed at two levels, at the level of a specific joint as well as the appendicular skeleton as a single unit (also referred to as the appendicular skeleton in toto).

Both inferential and descriptive analyses were performed, dependent on the number of observations within the dataset and subsequent power of the study.

3.2.5.1 Inferential Statistics

Logistic regression via a generalised linear mixed model with an underlying binomial distribution (GenStat, Version17, VSNi) was used to explore the association between the arthropathy status (negative, positive) of the appendicular skeleton in toto, and the following predictors: species, gender, age class, and their interactions. All modelling was performed at an age class level, with animal identification included as a random effect. Due to the high number of cheetahs in the study, and their dispersal between open-range and urban zoos, the effect of enclosure size on the prevalence of cheetah appendicular arthropathies was also investigated at this skeletal level. This was performed for both urban versus open-range zoos, and Institution A versus all other zoos, as described in Chapter 2. Interactions between cheetah enclosure size, age, and gender were also modelled. Due to smaller data sets for some appendicular joints (range 37-111 studies), a generalised linear model (GenStat, Version17, VSNi) was used for logistic regression analysis of the association between age, species, gender, and their interactions, and arthropathy status (negative, positive) for each of the six appendicular joint types. For these analyses, it was necessary to merge scoring of left and right joints, with the rule that if either joint was scored as arthropathy-positive during an age class, this constituted an overall positive score for that joint type. As with the generalised linear mixed model, modelling was performed at an age class level. Due to the combination of small data sets, and a low incidence of arthropathy-positive joints, it was not possible to analyse statistically for an association between arthropathy status and enclosure size in cheetahs for the six appendicular joint types.

All modelling was based on a binary output for arthropathy status (negative or positive). All predictors were categorical, and either nominal with several categories (species) nominal and dichotomous (gender, cheetah enclosure size) or ordinal (age class). Determination of the level of statistical significance, and further analysis of significant associations was done as described in Chapter 2.

3.2.5.2 Descriptive Statistics

Descriptive statistics were used to examine the prevalence, maximum severity, distribution patterns, features and nature of appendicular arthropathies. The majority of descriptive analyses were conducted according to age class, however age classes were pooled for smaller data sets. Pivot tables were utilised to generate tabulations, and where indicated, graphical depictions were generated to illustrate key findings.

The severity of appendicular arthropathies was analysed for each of the six appendicular joint types, and for the appendicular skeleton in toto. By utilising the severity score generated for each arthropathy-positive appendicular joint, the most severely affected joint type for each of the three species was determined. In contrast, the analysis of the severity of joint disease for the appendicular skeleton in toto necessitated a single severity score to be assigned to every study, irrespective of the number of appendicular joints captured. The highest severity score achieved at an individual appendicular joint level was also ascribed to the appendicular skeleton in toto for that study. This then allowed a comparison of arthropathy severity between the four age classes within species, and between the three species.

Investigation of the prevalence of features of appendicular arthropathies was conducted on data sets with pooled age classes, such that results represented an aggregated score from all radiographic findings for the appendicular joint, over the life of the animal. Features analysed were osteophytosis, enthesophytosis and/or dystrophic soft tissue mineralisation, increased intracapsular soft tissue opacity, subchondral bone sclerosis, subchondral bone lucency, and osseous bodies not consistent with meniscal ossicles. Each feature was scored as either present or absent, with a severity score of 1-3 recorded for osteophytes and enthesophytes. The three species were examined independently. Results report the percentage of arthropathy-positive joints, within joint type, that demonstrated each feature, with associated severity where applicable.

Unilateral versus bilateral distribution of appendicular arthropathies was evaluated for all appendicular joint types, with left and right joints of a given joint type treated as independent units. The analysis was conducted according to age class, with each animal restricted to one entry

per age class, and only animals with arthropathy-positive joint(s) were considered for inclusion. For an arthropathy-positive joint to be eligible for inclusion in the data set, the contralateral joint was required to be imaged and scored within the same age class. Each eligible arthropathy-positive joint type was then coded as having a unilateral or bilateral distribution for that animal in that age class. Results are reported as the percentage of 'animals in an age class' that demonstrated unilateral versus bilateral distribution of disease. These results are presented in three tables- a comparison of six joint types, a comparison of three species and a comparison of four age classes. A comparison of joint type within age class within species was not possible, due to fragmentation of already small data sets.

3.3 Results

3.3.1 Study Population

A total of 195 radiographic studies of 'animals within an age class', representing 146 individual animals (Figure 3.1) were analysed for the prevalence of arthropathies of the appendicular skeleton. Forty six of 195 (23.6%) studies were positive for arthropathy at one or more appendicular joints. The frequency distribution of modelled predictors against arthropathy status is shown (Appendix 3.1).

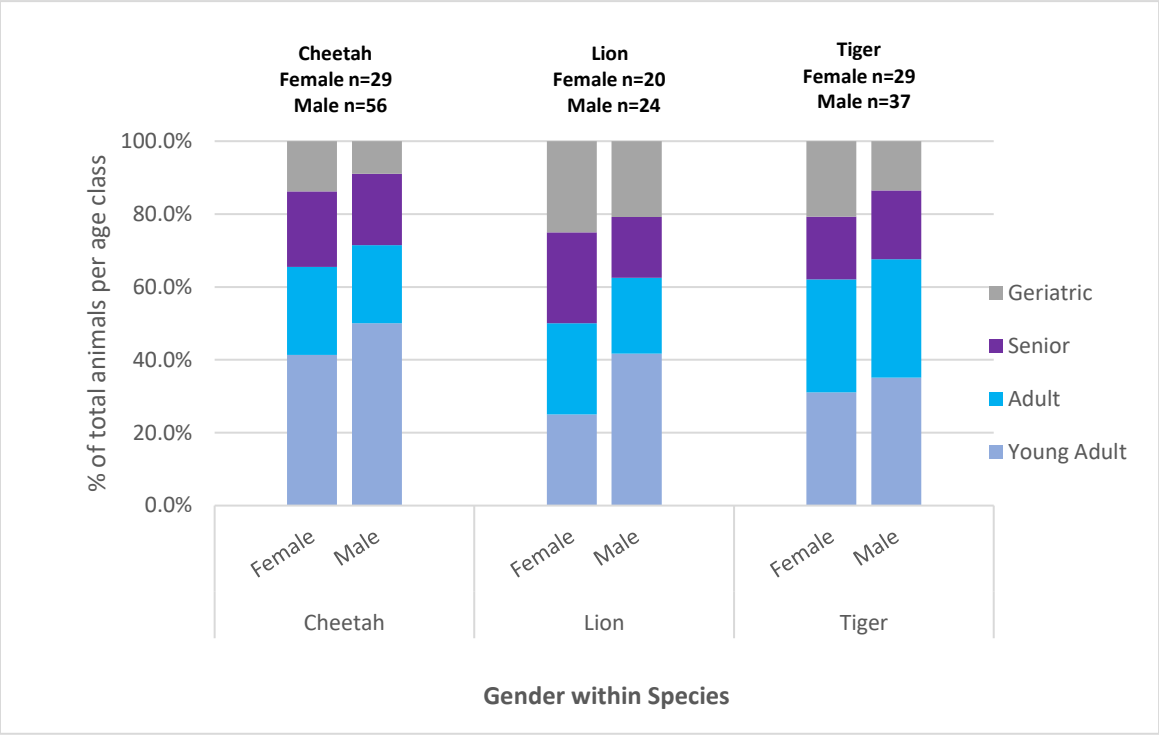

Figure 3.1 Distribution of 'animal within an age class' study population for analysis of arthropathies of the appendicular skeleton. n= number of individuals, gender within species

3.3.2 Modelling for predictors of arthropathy status

The only significant predictor for the detection of appendicular arthropathies was species (P=0.032). Cheetahs were predicted to have a significantly lower prevalence of arthropathies of the appendicular skeleton compared with lions and tigers, with means for predicted prevalence of 12.82% versus 30.97% and 30.93% respectively. The predicted means and standard errors for the differences, used for manual calculations of pair wise (LSD) calculations are reported (Appendix 3.2). P values from all modelling can be found in Appendix 3.3. The difference in observed prevalence of appendicular arthropathies between three species across four age classes is shown (Figure 3.2). No appendicular arthropathies were recorded for cheetahs from senior and geriatric age classes. In contrast, there were 12 young adult or adult cheetahs with appendicular arthropathies. Of these, seven were lost to follow up before moving into older age classes, three cases resolved, one case died, and one remains in the younger age classes as a current case.

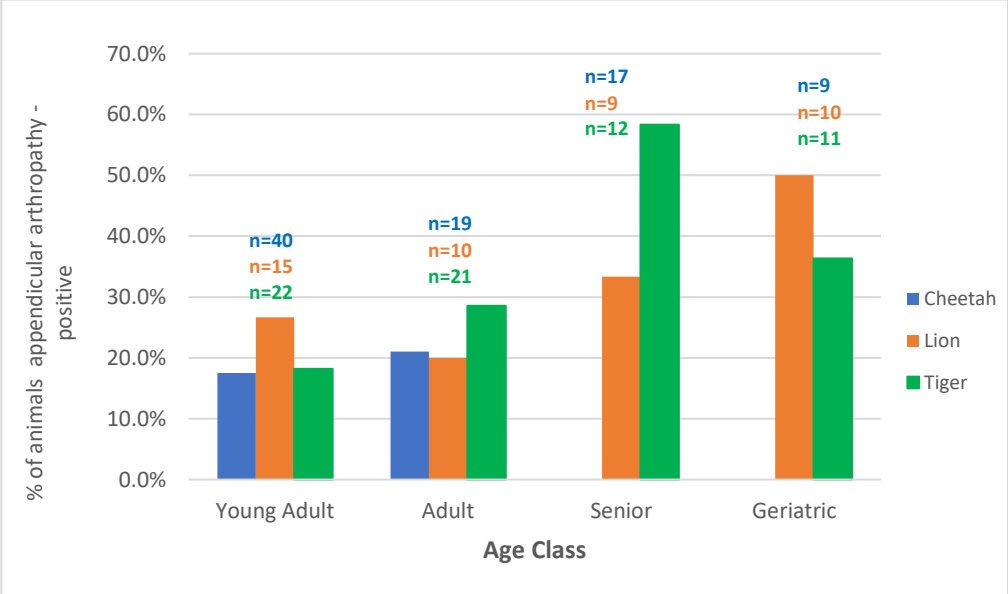

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Figure 3.2 Distribution of the prevalence of appendicular joint disease in cheetahs, lions and tigers as a function of age. n= number of cheetahs/lions/tigers in an age class

The observed prevalence of arthropathies of the six appendicular joint types for 'animals within age class' are shown (Table 3.1), with the frequency distribution of modelled predictors against arthropathy status for appendicular joint types presented in Appendix 3.4 a-f. 'Species' was the only significant predictor for arthropathies at a joint type level, with a significant association found for three of the six appendicular joint types, the shoulder (P=0.009), coxofemoral (P=0.013) and stifle (P=0.004) joints. Pairwise differences showed that lions had a significantly higher predicted prevalence of both shoulder and stifle arthropathies than cheetahs or tigers. In contrast, tigers showed a significantly higher prevalence of coxofemoral arthropathies than either cheetahs or lions (Figure 3.3). Predicted means with standard errors, and pairwise calculations, for all significant associations (Appendix 3.5 a-c) and P values for the modelling of associations between predictors and arthropathies of the six arthropathy joint types (Appendix 3.6) are presented.

Joint Type	**Total number**	**Percentage arthropathy positive**
Shoulder	37	16.2%
Elbow	64	18.8%
Carpus	63	6.4%
Coxofemoral joint	111	11.7%
Stifle	75	21.3%
Tarsus	53	9.4%

Table 3.1 Prevalence of arthropathies for six appendicular joint types for 'animal within age class', three species combined.

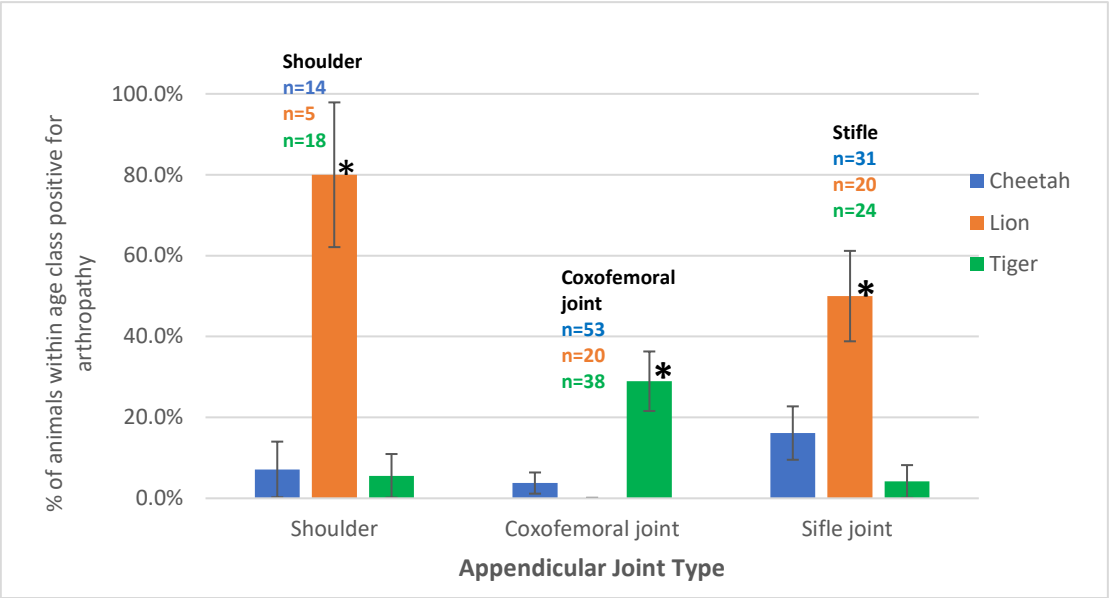

Figure 3.3 Prevalence of arthropathy-positive studies at three appendicular joints; shoulder, coxofemoral joint and stifle, for three species. n= number of joints included in the study, per species
* denotes significant difference between species.

3.3.3 The most frequently affected appendicular joint type

A total of 709 appendicular joints were scored for arthropathy status. With the exception of the tarsus in geriatric lions, the coxofemoral joint was the most frequently observed appendicular joint on the radiographs, in each of the four age classes for all three species. Sixty-nine individual appendicular joints, or 9.73% of all appendicular joints evaluated, showed radiographic features of arthropathy. The frequency of arthropathies at an appendicular joint type level, for animals within age class, for the three species is shown (Appendix 3.7 a-c).

The frequency of arthropathies for all appendicular joints of the cheetah was low, with a complete absence of appendicular joint pathology in the two older age classes. The shoulder was the most frequently affected appendicular joint in the young adult cheetah (2/10, 20%), closely followed the stifle (3/25, 12%). In the adult age class, the tarsus was the most frequently affected joint for the cheetah (2/10, 20%). The stifle and shoulder joints were the most frequently affected appendicular joints of lions (Figure 3.4a), recording a disease prevalence of 40% (14/35) and 50% (4/8) respectively. Stifle disease occurred at a consistent rate for adult, senior and geriatric lions.

The radiographically arthropathy-positive cohort of stifle studies consisted of 14 observations of 12 stifle joints from nine lions, with repeat observations restricted to a single lion with bilateral stifle pathology detected consecutively in senior and geriatric age classes. Thus, the consistent detection of stifle pathology across the three older age classes did not represent repeat observations on the same animals. Despite comparable or greater numbers of coxofemoral and tarsal joints captured, there was a notable absence of arthropathies in both of these joint types, for lions of any age. The elbow joint of older tigers was the most frequently affected joint for this species (Figure 3.4b). Eighty percent (4/5) of geriatric, and 30.1% of senior (4/13) tigers showed radiographic arthropathy of this joint. Singular cases of elbow disease were also detected in the two younger age classes. There were no repeat measures, with observations representing 10 different joints from eight tigers across the four age classes. Coxofemoral disease was also a feature for tigers, peaking at 31.25% (5/16) of joints imaged in the senior age class, 15% for the younger age classes, with the lowest frequency found in geriatric tigers (8.3%). Notably, and in contrast to lions, there was only a singular case of stifle disease detected in the tiger.

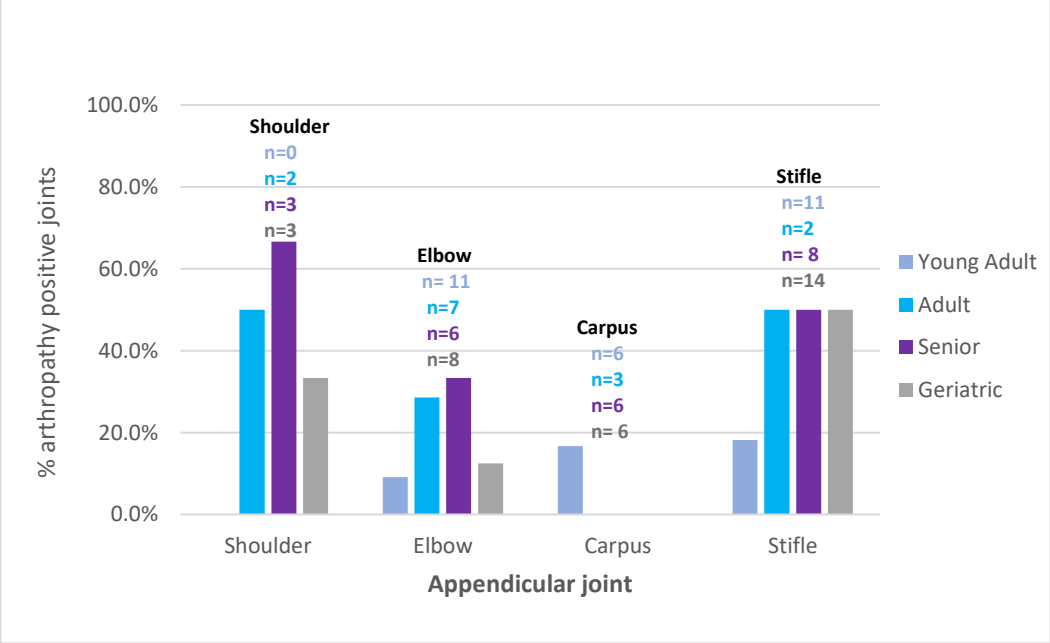

Figure 3.4a Prevalence of appendicular arthropathies in lions across four age classes. n= number of joints included in the study, per age class

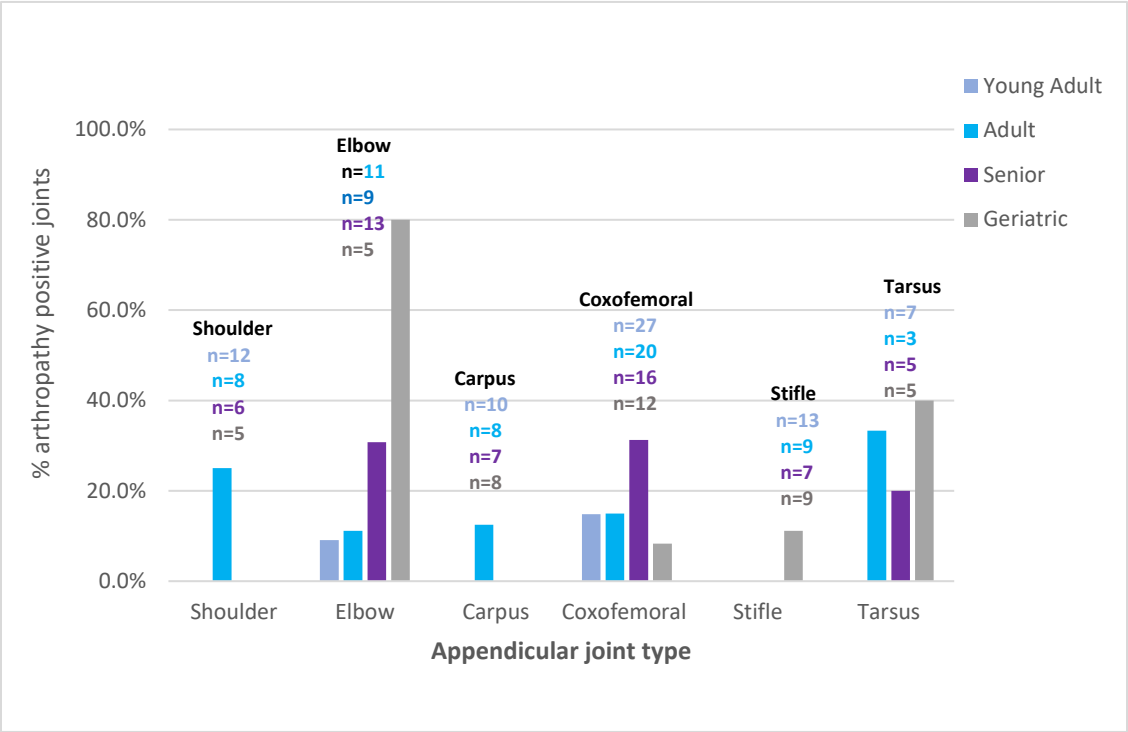

Figure 3.4b Prevalence of appendicular arthropathies in tigers across four age classes.
n= number of joints included in the study, per age class

3.3.4 Severity of appendicular arthropathies

The most severely affected appendicular joints in the cheetah were equally the shoulder and the tarsus, with an average maximum severity 2.5 (moderate -severe). The most severely affected joints for lions were equally the shoulder and elbow, with an average maximum severity of 1.67 (mild-moderate). The joints most severely affected in the tiger were the shoulder (average maximum severity 3, severe) followed by the tarsus with an average maximum severity 2.25 (moderate-severe), however the number of affected joints was low, with only two cases of shoulder and four cases of tarsal arthropathies. In comparison, tiger coxofemoral and elbow joints, each having 10 affected joints, scored an average maximum of severity of 1.8 and 1.7 respectively (mild-moderate). Tigers with tarsal arthropathy were on average older than those with coxofemoral or elbow disease. Tabulations of severity data for the six appendicular joint types for each of the three species are presented (Appendix 3.8 a-c).

To examine the effect of age, the severity of appendicular arthropathies was also examined for the four age classes, at the level of the appendicular skeleton in toto (Figure 3.5). Tigers were the only species to show increased appendicular arthropathy severity with age, and overall, the highest severity grades were seen in geriatric tigers.

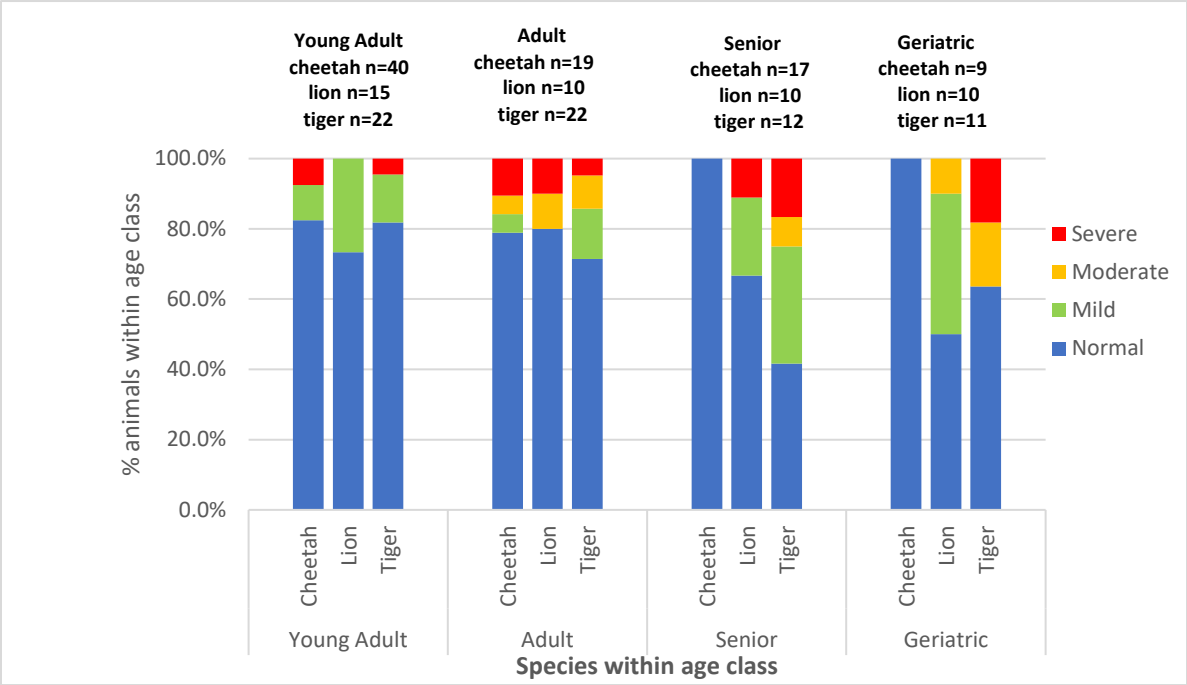

Figure 3.5 Distribution of maximum severity of arthropathy of the appendicular skeleton for the cheetah, lion and tiger across four age classes. n= number individuals per species, within an age class

In addition, the prevalence versus severity of disease was compared for each of the six appendicular joint types, according to age class and species (Table 3.2). Due to the low prevalence of appendicular arthropathies in the cheetah, the table is confined to the lion and tiger. This table was compiled with the zoo clinician in mind, to serve as a guideline for the expected distribution and severity of appendicular joint disease. Results highlight the low prevalence of disease in young adults of both species and the differing distribution and severity of disease between the lion and tiger with age.

		Shoulder	Elbow	Carpus	Coxofemoral	Stifle	Tarsus
Lion	Young Adult						
	Adult	XX	X			XX	
	Senior	XX	X			XX	
	Geriatric	X				XX	
Tiger	Young Adult						
	Adult	X					X
	Senior		X		X		
	Geriatric		XXX				X

Table 3.2 Prevalence and severity of arthropathies at an 'appendicular joint type' level for the lion and tiger. Prevalence: X ≥25%, <50%; XX ≥50%- <75% ; XXX ≥ 75%. Severity: Green, mild, average severity 1.0-1.5; Yellow, moderate, average severity score 1.6-2.4; Red, severe, average severity score ≥ 2.5.

3.3.5 Features of Appendicular Arthropathies

There were insufficient numbers of arthropathy-positive joints to draw firm conclusions regarding the relative frequency and spectrum of changes for the six different appendicular joint types for the three different species. However, osteophytosis was the most frequently identified radiographic feature and was found in 64.5% (40/62) of all pathological appendicular joints. Osteophytosis was most severe in the shoulder joint (Figure 3.6a), and tigers were most prone to this feature. Enthesophytosis and/or dystrophic soft tissue mineralisation was found in 48.4% of all joints (Figure 3.6b), and most commonly identified in the tiger, in particular elbow and tarsal arthropathies. The most severe enthesophytosis was seen in both the coxofemoral and tarsal joint in this species, with the average severity scored as mild to moderate. Degenerative disease confined to enthesophytosis or dystrophic soft tissue mineralisation was unusual, accounting for only eight of 62 or 12.9% of all diseased joints. Two of eight cases of subchondral bone lucency occurred in the absence of sclerosis, and were categorised as osteochondrosis (OC) lesions of the shoulder and elbow joint. Increased intracapsular soft tissue opacity (a change consistent with joint effusion) was uncommon, detected in only 8/62 or 12.9% of all diseased joints and was most frequently recognised in the cheetah. The radiographic features most commonly identified in both the elbow and coxofemoral joint in all three species were osteophytosis and

enthesophytosis/dystrophic soft tissue mineralisation. The stifle showed the greatest spectrum of radiographic features in the cheetah and lion (Figure 3.6c). Additionally, for six of the 12 diseased stifle joints of lions, radiographic changes were confined to the femoropatellar joint. Tigers recorded the most frequent and the most severe degenerative features (Figure 3.6d). This finding applied to all appendicular joint types. A tabulation of selected features of appendicular arthropathies, at an individual joint level, for all arthropathy positive appendicular joints, is shown (Table 3.3).

3.3.6 Unilateral versus bilateral distribution of appendicular arthropathies

Forty-three appendicular joint sites from 31 animals met eligibility requirements for this analysis. Three animals had an abnormal joint appear in multiple age classes. In all cases the joint was found to be static in appearance, without progression of disease as the animal aged. These joints were graded as a finding in each of the respective age classes. Unilateral disease was more common for all joint types, with 67% of all arthropathies unilateral in distribution. The elbow showed the highest prevalence of bilateral disease, followed by the shoulder and stifle. Bilateral disease was more common in older age classes, and the lion was most likely to have bilateral joint disease (40%) compared with the tiger (31.6%) and cheetah (22.2%). Bilateral disease was most commonly seen in senior lions and geriatric tigers (Appendix 3.9 a-d).

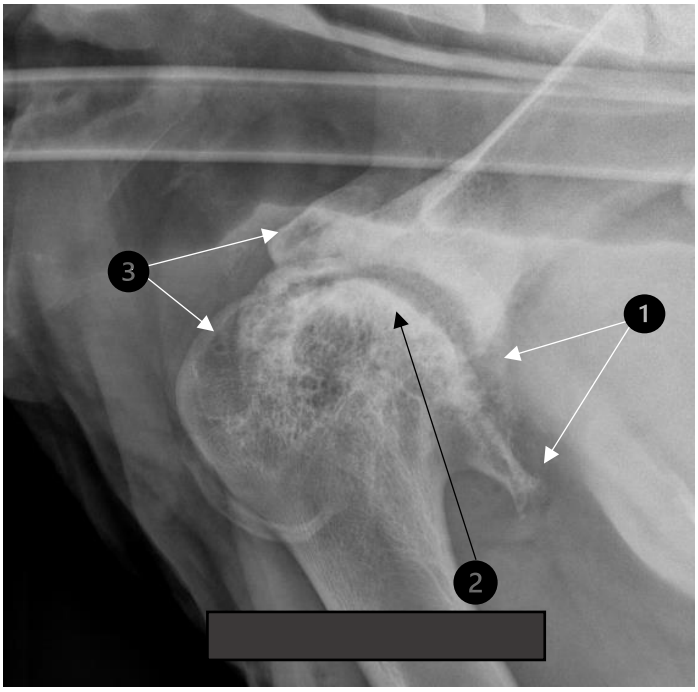

Figure 3.6a Mediolateral radiograph of the shoulder of an adult (10 y) male lion showing severe osteophytosis (1), subchondral bone sclerosis (2), and subchondral bone lucency (3).

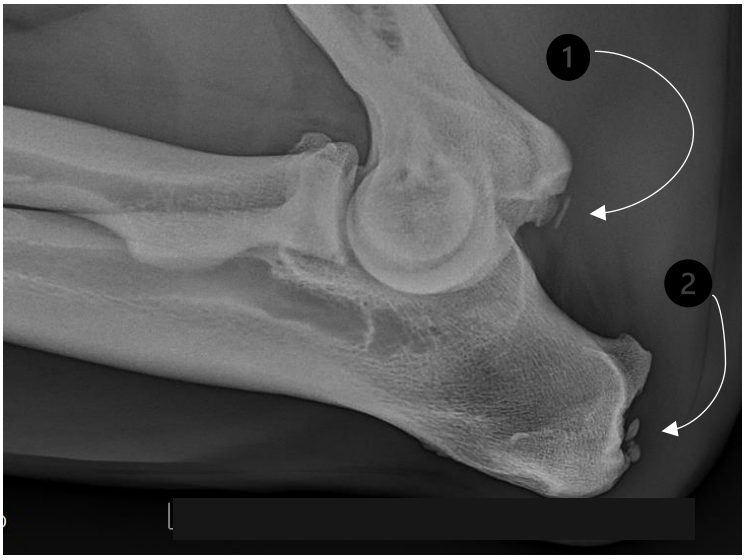

Figure 3.6b Flexed mediolateral radiograph of the elbow of a senior (13 y) female tiger with radiographic features of arthropathy confined to enthesophytosis, both joint associated (1) and remote to the joint (2).

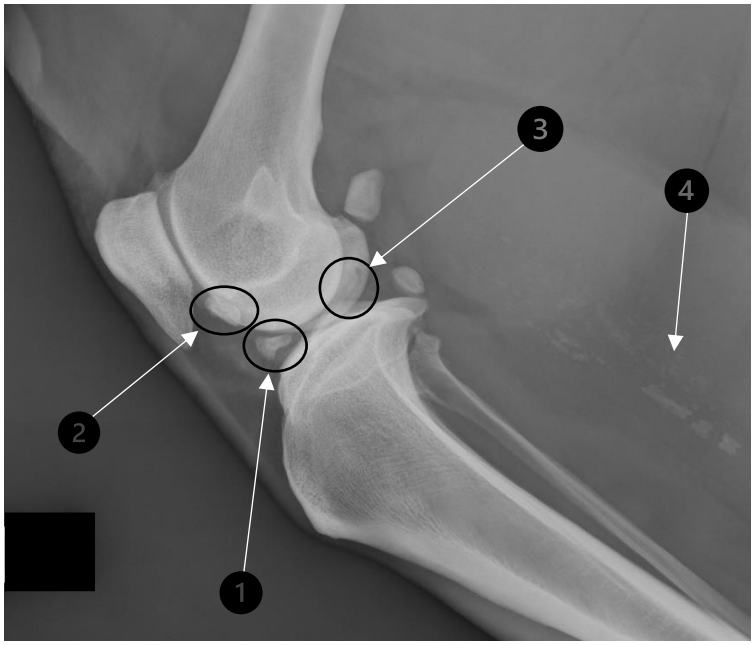

Figure 3.6c Mediolateral radiograph of the stifle of a geriatric (17 y) lioness showing two intraarticular osseous bodies, consistent with meniscal ossicle (1), and joint mouse (2), subchondral bone crescent-shaped lucency with sclerotic zone (3), and dystrophic mineralisation of soft tissue in crus (4).

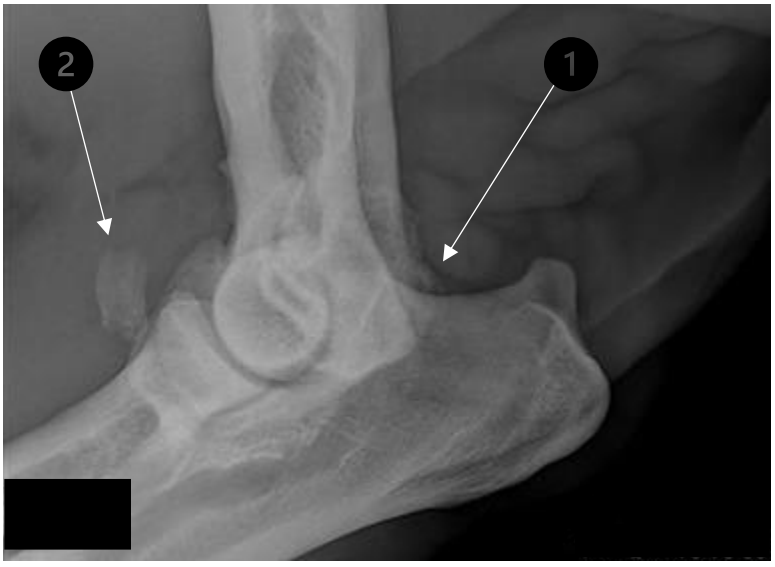

Figure 3.6d Mediolateral radiograph of the elbow of a senior (14 y) male Bengal tiger showing severe osteophytosis (1) and enthesophytosis (2).

Species	SITE	Total	Osteophytosis		Enthesophytosis and dystrophic soft tissue mineralisation		Intra capsular soft tissue opacity		Subchondral bone sclerosis		Subchondral bone lucency		Osseous body(s)-non meniscal ossicles	
Cheetah	Shoulder	2	1	50%	0	0%	0	0%	1	50%	2	100%	0	0%
	Carpus	2	1	50%	0	0%	0	0%	0	0%	0	0%	0	0%
	Coxofemoral	3	1	33%	2	67%	0	0%	1	33%	1	33%	0	0%
	Stifle	3	2	67%	1	33%	3	100%	1	33%	0	0%	1	33%
	Tarsus	2	1	50%	0	0%	2	100%	0	0%	0	0%	2	100%
	Cheetah Total	**12**	**6**	**50%**	**3**	**25%**	**5**	**42%**	**3**	**25%**	**3**	**25%**	**3**	**25%**
Lion	Shoulder	3	3	100%	1	33%	0	0%	1	33%	1	33%	0	0%
	Elbow	6	4	67%	4	67%	0	0%	1	17%	0	0%	1	17%
	Carpus	1		0%	0	0%	0	0%	0	0%	0	0%	1	100%
	Stifle	12	7	58%	6	50%	3	25%	2	17%	1	8%	3	25%
	Lion Total	**22**	**14**	**64%**	**11**	**50%**	**3**	**14%**	**4**	**18%**	**2**	**9%**	**5**	**23%**
Tiger	Shoulder	2	2	100%	2	100%	0	0%	2	100%	1	50%	0	0%
	Elbow	10	6	60%	7	70%	0	0%	1	10%	1	10%	2	20%
	Carpus	1	1	100%	0	0%	0	0%	0	0%	0	0%	0	0%
	Coxofemoral	10	8	80%	4	40%	0	0%	3	30%	0	0%	0	0%
	Stifle	1	1	100%	0	0%	0	0%	0	0%	0	0%	0	0%
	Tarsus	4	2	50%	3	75%	0	0%	1	25%	1	25%	1	25%
	Tiger Total	**28**	**20**	**71%**	**16**	**57%**	**0**	**0%**	**7**	**25%**	**3**	**11%**	**3**	**11%**
Grand Total		**62**	**40**	**65%**	**30**	**48%**	**8**	**13%**	**14**	**23%**	**8**	**13%**	**11**	**18%**

Table 3.3 Prevalence of selected features of appendicular arthropathies, at an individual joint level, for the cheetah, lion and tiger, age classes combined.

3.3.7 Nature of arthropathies of the appendicular skeleton

Seventy-two appendicular joints from 38 animals were eligible for assessment of nature of arthropathy (Appendix 3.10 a-b). Five animals were present in more than one age class**,** and 17 contributed more than one appendicular joint within any one age class. Of the 72 appendicular joints, five were repeat measures, occurring in more than one age class, with four joints appearing in two consecutive age classes, and one joint in three consecutive age classes. Eighty percent (57/72) of appendicular arthropathies were considered degenerative and 20.5% (15/72) nondegenerative. The tiger was the only species to show a progressive increase in degenerative arthropathies with age, recording 60%, 77.8%, 90.9% and 100% for young adult, adult, senior and geriatric age classes respectively. However, older (senior and geriatric) lions recorded a higher percentage of degenerative arthropathies (94.1%) than younger (young adult and adult) lions (75%). No inciting cause was identified for 62.7% (37/57) of all degenerative appendicular arthropathies. Of the 20 degenerative appendicular joints where a causal factor was reported, five cases were secondary to trauma, one case of stifle DJD was suspected to be the result of a partial tear of the caudal cruciate ligament (based on necropsy findings), 13 cases were associated with presumed or confirmed developmental disease, and one case was secondary to a septic arthropathy. Developmental lesions most frequently associated with subsequent radiographic degenerative changes were hip dysplasia (HD) and OC/ OCD. Suspected or confirmed HD was confined to tigers, with five coxofemoral joints from four tigers affected. Radiographic characteristics suggestive or consistent with OC lesions were identified in all three species, and confirmed in the lion (shoulder, necropsy), cheetah (tarsus, surgery) and tiger (elbow, advanced imaging). An intraarticular osseous body associated with a zone of radiographic lucency, consistent with OCD, was identified in the stifle joint of a lion but no further confirmatory diagnostic procedures were undertaken. Of the 15 nondegenerative appendicular arthropathies, five were developmental, six were traumatic, two were septic and two cases of intraarticular osseous bodies in the absence of other disease were classified as unclear. Three developmental (OC/OCD), and two traumatic arthropathies were subsequently detected to transition to radiographic DJD over time. Details regarding the nature of all appendicular arthropathies are presented as three species-specific tables in Appendix 3.11 a-c.

3.4 Discussion

This study is the most comprehensive review of appendicular DJD in large cats to date. From the previous, and comparable study of the axial skeleton (Chapter 2), it was determined that captive large cats commonly experienced spinal DJD. This study showed that appendicular DJD is also common, with approximately 25% of all studies showing joint disease at one or more locations within the appendicular skeleton, and 80% of joint disease degenerative in nature. Whilst multiple variables were examined for an association with joint disease, 'species' was the only factor that explained the variation in the data. This was the case for both the appendicular skeleton in toto, and for appendicular joint types, with both lions and tigers showing significantly more joint disease than cheetahs.

3.4.1 Patterns of degenerative arthropathies in the lion, tiger and cheetah

Increased levels of joint disease, almost exclusively degenerative in nature, were detected in older (senior or geriatric age class) lions and tigers, and an underlying aetiology was only rarely identified. This is not unusual. For the few reported case studies of appendicular osteoarthritis (OA)/DJD in captive lions and tigers, no inciting causes were either confirmed,[9] or reported,[8; 11; 12] and studies of radiographic appendicular DJD in the domestic cat similarly struggle to associate radiographic changes with an identifiable inciting cause.[14; 15; 17] Whilst disease prevalence was similar for the lion and tiger, radiographic severity differed, with the tiger alone showing increased severity with age. Thus, DJD in older tigers was typically more severe than that seen in older lions, with the exception of the elbow joint, which could be severely affected in both species. Increasing age also revealed the pattern of disease distribution. This pattern differed markedly between the two species in the older age classes.

Although in the older lion both the shoulder and stifle showed a similar prevalence of disease, there was a notable disparity in the number of images available for these two joint types, with comparatively few shoulder images, relative to the robust number of stifles captured. This can be explained by the inherent difficulties of imaging the shoulder joint in these large, heavy patients, with both accurate positioning and X-ray beam penetration of the joint challenging. Consequently, imaging is often reserved for those patients with a strong clinical imperative, and

this may have introduced bias regarding the prevalence of shoulder disease reported in this study. Thus, disease of the stifle joint was considered the key finding for the lion. Radiographic changes were typically mild, and 50% of all cases had disease restricted to the femoropatellar joint. Although almost all cases had no known attributable cause established, some underlying disorders were identified, including confirmed caudal cruciate rupture (arthroscopic confirmation) and radiographic OCD. The distribution and radiographic features of appendicular degenerative arthropathies for the captive-held lion documented in this study are very similar to that reported for wild lions. In a radiographic study of 15 free-ranging lions (age range 16 months-12 y, average age 6 y) degenerative changes, although generally considered incidental, were thought to be most likely age-related or posttraumatic.[38] This raises the question as to whether similar factors influence DJD development in captive versus wild lions.

The distribution of DJD in tigers differed from that of lions. Only a single case of stifle disease in older tigers was identified whereas a high prevalence of coxofemoral DJD, and tarsal disease, which was often severe, was documented. Another key finding was an overwhelming bias for degenerative elbow disease in geriatric tigers. As with the other joints, elbow disease ranged from mild to severely affected, with a causative factor identified in only one case. The relatively lower prevalence of elbow disease in the younger age classes suggests that the tiger elbow appears susceptible to degenerative changes associated with advancing age. This trend was not appreciated for any other joint type in any of the three species. In particular, for both the tiger and lion, DJD prevalence for other joint types either peaked at, or plateaued beyond, the senior age class. Whether this finding reflects increased morbidity and therefore earlier mortality associated with DJD of joints other than the elbow, is unclear. However, if this is the case, this may explain the unexpected finding of only a single case of coxofemoral disease in the very oldest of tigers, despite a steadily rising prevalence in the preceding age classes.

In contrast to older lions and tigers, this ageing trend was not apparent in the cheetah. Cheetahs were unexpectedly exceptional in their complete absence of radiographically-detectable joint disease in older animals. This finding was more remarkable for the fact that a greater number of

appendicular joints were captured for older cheetahs than for either of the other two species, in the equivalent age classes.

Conversely, joint disease was found in younger (young adult and adult) cheetahs, with DJD and nondegenerative arthropathies occurring at a similar frequency. The appendicular joints of younger cheetahs were found to be susceptible to a range of insults, including developmental disease, trauma and sepsis. These causes were associated with severe radiographic changes and contributed to the finding that an inciting cause could be identified for almost all cases of DJD detected in younger cheetahs. In this respect younger cheetahs were similar to younger lions and tigers. A comparable disease prevalence was recorded for all three species for these younger age classes, and the lion and tiger too were susceptible to a variety of causative factors that resulted in disease, with degenerative changes also identified.

However, whilst for the lion and tiger joint disease in younger age classes was associated with an increasing prevalence of DJD with age, the absence of DJD in older cheetahs, despite identification in younger animals, is difficult to reconcile, as it is well accepted that DJD is an irreversible disease state and therefore does not 'disappear' with age. There are two aspects to address when considering how best to explain these contradictory results. These findings would suggest that cheetahs with younger onset OA/DJD do not re-present as older, DJD-positive cases. One possible explanation is that, whilst original joint insults and early DJD affecting younger cheetahs may be painful, the clinical impact of early-onset DJD may diminish with age. In both human and companion animal medicine the clinical expression of DJD is appreciated to be both complex and poorly understood, with a range of factors and comorbidities influencing clinical impact.[39; 40] However, it is recognised that the disease fluctuates, clinical signs can be intermittent, and that DJD may be quiescent.[41-44] It is therefore feasible that early-onset DJD may be subclinical in older cheetahs, and if so, this will have influenced the likelihood of recapture of radiographic disease in this study. As this was not a radiological survey where all joints were imaged, but rather a retrospective study with opportunistic data acquisition, with the exception of coxofemoral joints, appendicular joints were most often imaged because there was a clinical indication. If

older cheetahs did experience subclinical DJD, there would be no clinical indication for imaging. This may in part account for the absence of DJD in older cheetahs reported here.

Equally, a failure to detect appendicular DJD in the older cheetah suggests that, unlike lions and tigers, cheetahs have a decreased susceptibility to the more typical age-related OA/DJD. Whilst OA/DJD development and progression is considered complex and multifactorial, with individual susceptibility most likely an interaction between biomechanical, environmental, systemic and genetic factors,[45] it is generally acknowledged that OA /DJD-related changes represent an accumulation of microtrauma over a lifetime.[46] Why cheetahs may have a lowered predisposition to age-related OA/DJD is speculation, however examination of osteopathological specimens has suggested that DJD of large cats is related to body size.[7; 47] Collectively, Panthera are a very different body type compared with the cheetah, who, although considered a 'large cat', are a much smaller unit. Although size alone could account for the difference in patterns of joint disease seen in this study, this simplistic approach does not consider the heterogeneity of OA. Joints impacted may differ between species, dependent on both how they are used, and the forces they are subjected to. Although force will be impacted by weight, species-specific musculoskeletal adaptations to meet niche ecological requirements may also play a role, and therefore function, design and ecology all need to be considered. Studies have shown that, whilst musculoskeletal differences are recognised, the lion and tiger are morphologically very similar, compared to the specialised cheetah.[19; 22; 26-31; 48-51] Forelimb adaptations in the cursorial cheetah confer superior shock absorption, whereas supination and pronation are needed for the 'stalk and ambush' lion and tiger. Musculoskeletal differences of the elbow joint [30] are reported to account for the pendulum motion of the cheetah forelimb, as opposed to the enhanced forelimb abduction and adduction [26] and free rotary motion [52] characteristic of lion and tiger gaits respectively. Given the notable prevalence of elbow DJD for the lion and tiger, compared with a complete absence in the cheetah, these findings are particularly pertinent. However, whether any of these anatomic or biomechanical differences translate to either altered joint architecture or a predisposition for degenerative disease requires further investigation.

In summation, several key points are identified from this study's findings regarding degenerative arthropathies of the appendicular skeleton in younger versus older cheetahs. Firstly, DJD, often due to a range of identifiable aetiologies, was demonstrated in younger cheetahs, confirming that DJD does exist in this species. That younger cheetahs were imaged suggests that DJD at this time was clinically significant. In contrast, the complete absence of radiographic appendicular DJD in older cheetahs in this study implies that, unlike lions and tigers, cheetahs are not susceptible to age-induced 'wear and tear' DJD. Equally, the failure to identify radiographic DJD in the older cohort signifies that older cheetahs with younger-onset OA/DJD were not captured by the study. This finding suggests that, as these affected cheetahs age, their DJD may become subclinical. The clinical implications of these combined findings are that firstly, if cheetahs can avoid DJD as younger animals, they may be unlikely to develop DJD as older animals, and likewise, if DJD is detected radiographically in an older cheetah, this study's findings would suggest that the degenerative processes may have begun when the animal was considerably younger.

3.4.2 Radiographic features of degenerative arthropathies

Osteophytosis was the most frequently identified radiographic feature of joint degeneration in captive lions, tigers and cheetahs. Whilst not pathognomonic for OA, osteophytosis is widely recognised as one of the most useful markers for OA diagnosis [44] and was evident in all joint types and displayed by all species. Although occasionally graded as severe, most osteophytosis was classified as mild and associated with small osteophytes only. This finding has significant implications. Different species have been shown to produce osteophytes to a greater or lesser extent as part of their degenerative process. The consensus is that the domestic cat does not form radiographically detectable osteophytes as readily as the dog,[15; 17; 53] showing typically mild osteophytosis on imaging, and that, with the exception of the coxofemoral joint, osteophytosis is not the most frequently recognised feature of OA/DJD in this species.[16] The similarly mild osteophytosis of large cats reported here suggests that the radiographic changes of DJD demonstrated in these species are also often subtle and may be easily missed. However, whether this translates to an under-detection of OA in large cats remains to be clarified.

Not all joint-associated mineralisation was consistent with osteophytosis. The most common alternative identified was enthesophytosis, seen in a range of joint types, with the lion and tiger particularly affected. Enthesophytosis can be difficult to differentiate from osteophytosis radiographically, with the distinction based on the anatomical landmarks of the articular cartilage: bone interface versus insertion points of joint-associated tendons and ligaments.[54] However, the distinction is important, as these two features may be associated with very different clinical significances. With only rare exceptions, the presence of osteophytosis can be considered to denote OA, and therefore carries with it the myriad of clinical ramifications of this disease, both immediate and future. In contrast, whilst enthesophytosis does represent a degenerative change of the involved ligament or tendon, the clinical significance is incompletely understood, and in humans, enthesophytes may be considered both an incidental finding and a phenomenon of ageing.[55]

3.4.3 Nondegenerative arthropathies of the appendicular skeleton

The scale and scope of this study also allowed for an appreciation of the location and nature of developmental lesions of the appendicular joints of captive cheetahs, lions and tigers. Two developmental diseases particularly featured. The first, and the most commonly detected developmental joint disease, was OC/OCD. OC/OCD has only rarely been reported in large cat species, with published papers confined to case series of stifle OCD in snow leopards [56; 57] and distal ulnar metaphyseal OC in cheetahs.[58] This study identified radiographic characteristics consistent with OC/OCD in all three species, with detection in the elbow (tiger), shoulder (cheetah and lion), stifle (lion) and tarsus (cheetah). As can best be ascertained, this study is the first to formally report radiographic OC/OCD lesions at these sites in these three species. Whilst the radiographic demonstration of an associated intraarticular osseous body will allow a diagnosis of OCD as opposed to OC,[36; 37] this was only possible in one of the cases identified here. An important differential diagnosis of radiographic intraarticular osseous bodies, particularly if in the absence of subchondral bone lucency characteristic of OC, is synovial osteochondromatosis. Synovial osteochondromatosis has been described in the tiger elbow [11] and has been reported in the domestic cat.[59; 60] Although the radiographic appearance can be suggestive, diagnosis invariably requires histopathological examination of biopsy specimens. As a radiological study,

this investigation did not definitively identify cases of synovial osteochondromatosis, however it remained a differential diagnosis for several animals.

This study is also the first to describe cases of coxofemoral DJD consistent with the developmental disease hip dysplasia (HD). All cases were confined to the tiger, with two young (10 months) tigers either confirmed or consistent with this diagnosis. Whilst coxofemoral DJD in older (7.5 y, 10 y) tigers has been reported in the literature, there was no mention of HD as the inciting cause.[8; 9]

Finally, a single case of medial patella luxation with severe secondary stifle OA in a cheetah was found in this study. This is the second reported case of this lesion in a cheetah. In the first case the aetiology of the patellar luxation could not be determined,[10] but the relatively young age at detection for the case identified in this study was consistent with a congenital or developmental aetiology.

This study's findings of developmental disease in captive large cats may have further implications. It is notable that the appendicular joint types predisposed to OC/OCD in the lion and tiger, and the confirmation of HD in the tiger, partially reflect the relative prevalence and distribution of DJD in these two species as they age. It is widely accepted in companion animal medicine that OC/OCD and HD can both be difficult to diagnose on plain radiography, and subsequently result in degenerative changes to the affected joint.[37; 61-63] This is consistent with the findings of this study, where over 80% of all OC/OCD lesions were associated with subsequent degenerative changes. It is therefore possible that undetected developmental disease in younger lions and tigers could contribute to the prevalence and distribution of degenerative disease found in the older cohort.

3.4.4 Recommended protocol for the radiographic detection of appendicular joint disease

The different patterns of joint disease identified for the three species have important implications for the clinician when planning survey radiography for disease detection. This is particularly pertinent as radiography of all appendicular joints is seldom possible, with prioritisation of time

and resources often necessary. Consequently, dependent on the species, vastly different recommendations are offered.

As this study has identified a range of susceptible joints in both the older lion and tiger, the following should be incorporated into routine health screening in older animals. The stifle joints of all lions five years of age and older should be assessed, with special attention to the femoropatellar joint, and evaluation for both cruciate ligament disease and pre-existing OC/OCD. As the shoulder was also a site of concern for this species, with a high prevalence of DJD which could be radiographically severe, wherever possible, this joint also should be included. In addition, as elbow disease was detected in the lion, with a similar range of severity to the shoulder, imaging of the elbow is also recommended. Radiography is indicated for both the elbow and coxofemoral joints of older tigers, with added emphasis for elbow imaging of geriatric animals due to the high prevalence of disease. However, due to the possibility of developmental disease, imaging of both the elbow and coxofemoral joint at all age classes is warranted. Although DJD prevalence was lower in the tarsus and shoulder for the tiger, disease was severe and therefore whilst survey radiography may not be indicated, the clinician should be aware of the potential for significant disease at these sites.

The radiographic protocol recommended for older cheetahs is dependent upon the purposes of imaging. As this study found no evidence of clinically significant appendicular DJD in older cheetahs, routine imaging of joints in these animals does not appear to be indicated, with radiography reserved for those cases with a clinical suspicion. However, the findings of disease in young cheetahs would suggest that DJD may be detectable in the older cohort. Therefore, if conducting a health and disease survey, radiographic screening of a range of appendicular joints in the older cheetah is recommended.

For younger animals of all species, where clinically indicated, attention should be directed at those sites where developmental disease has been identified: the stifle and shoulder of lions (OC/OCD), elbow (OC) and coxofemoral joints (HD) of tigers, and the carpus, tarsus, and shoulder (OC/OCD), and stifle (medial patella luxation) of cheetahs. As an inciting cause was identified for almost all radiographic DJD in younger cheetahs, investigation of possible aetiologies in these

cases would appear worthwhile. In particular, where trauma has been ruled out, sepsis and developmental disease should be considered.

Although most animals showed unilateral disease, bilateral disease was detected in one third of all cases, underpinning the recommendation that the contralateral joint be imaged for all arthropathy-positive appendicular joints. This particularly pertains to the elbow, which showed the highest prevalence of bilateral disease. In addition, bilateral disease should be increasingly expected as the animal ages, and this is particularly applicable to ageing lions. Regarding radiographic projections, whilst orthogonal projections are preferred for all appendicular joints, it was this study's observation that for the shoulder and stifle a standard mediolateral view, and for the coxofemoral joint an extended ventrodorsal view, provided sufficient opportunity to assess for radiographic features of joint disease. In contrast, orthogonal projections of the elbow are warranted, including both neutral and flexed mediolateral views.

3.4.5 Future Studies

Larger, prospective investigations with comprehensive radiography of appendicular joints are needed to further define this study's findings. Long-term and repeat imaging of not only developmental disease, but all nondegenerative aetiologies will assist in clarifying any association with progression to degenerative disease later in life. The use of advanced imaging techniques, in conjunction with gross inspection and histopathological studies of joints, is encouraged, and will be needed to fully appreciate the extent of joint disease in the appendicular skeleton of these species, and the contribution of DJD within this spectrum. The similarities between this study's findings and those reported for free-ranging lions does raise the question as to what impact the conditions of captivity may have for appendicular DJD in this species. Whether comparable similarities exist between free-ranging and captive tigers, or cheetahs, is currently unknown, however clearly this is an area that requires further research as a priority.

3.5 Conclusion

This radiological study, the first to investigate appendicular joint disease in captive cheetahs, lions and tigers, provides a comprehensive overview of the spectrum of arthropathies detected in these species. Appendicular arthropathies were found to be both common, and most commonly degenerative in nature. However, despite similarities as younger animals, the three species showed markedly differing patterns of disease as they aged. Whereas the lion and tiger showed a more typical age-related increased prevalence of DJD, the conspicuous absence of DJD in the older cheetah was an unexpected finding and raises the question as to whether this species is less susceptible to DJD in advancing age. In addition, whether early-onset DJD remains clinically significant later in life in this species requires clarification.

A clear species-specific site predisposition for joint disease was identified for both the lion and tiger, with stifle disease identified as a priority for further investigation in the lion. In contrast, although coxofemoral pathology was a feature for the tiger, it was the prevalence and severity of elbow disease that was remarkable. Indeed, tigers as a species were notable for displaying the most severe radiographic appendicular DJD of the three species. This study has also showed that captive large cats are susceptible to a range of insults that result in appendicular joint disease, including trauma, sepsis and developmental disease. Many of these developmental diseases are reported here for the first time in these species.

Appendix 3.1 Frequency distribution of predictors against arthropathy status; appendicular skeleton.

Predictor	Sub-category	Total Count	Arthropathy Negative		Arthropathy Positive	
			Number	Percent	Number	Percent
Species	Cheetah	85	74	87.1%	11	12.9%
	Lion	44	30	68.2%	14	31.8%
	Tiger	66	45	68.2%	21	31.8%
Gender	Female	78	63	80.8%	15	19.2%
	Male	117	86	73.5%	31	26.5%
Age Class	Young Adult	77	62	80.5%	15	19.5%
	Adult	50	38	76.0%	12	24.0%
	Senior	38	28	73.7%	10	26.3%
	Geriatric	30	21	70.0%	9	30.0%
Grand Total		**195**	**149**	**76.4%**	**46**	**23.6%**
Cheetah Enclosure Size						
Urban vs Open Range	Open Range	48	42	87.5%	6	12.5%
	Urban	37	32	86.5%	5	13.5%
Institution A vs Other	Institution A	19	15	79.0%	4	21.1%
	Other	66	59	89.4%	7	10.6%
Grand Total		**85**	**74**	**87.1%**	**11**	**12.9%**

Appendix 3.2 Predicted (non back transformed) means and standard errors of differences, used for manual calculation of pairwise differences for significant predictors of arthropathies for the appendicular skeleton.

Species	**Predicted Means**
Cheetah	-1.917
Lion	-0.802
Tiger	-0.803
Standard errors of differences	
Average:	0.482
Maximum:	0.5058
Minimum:	0.4621

Appendix 3.3 P values from modelling of predictors against arthropathy status for the appendicular skeleton. Significant associations are highlighted in red.

Predictor	**P value**
Age	0.755
Species	**0.032**
Gender	0.191
Age Species Interaction	0.925
Species Gender Interaction	0.778
Age Gender Interaction	0.497

Cheetah Enclosure Size	Urban vs Open Range	Institution A vs Other
Enclosure Size	0.848	0.273
Enclosure Size Age Interaction	0.98	0.844
Enclosure Size Gender Interaction	0.996	0.454

Appendix 3.4 a-f Frequency distribution of predictors against arthropathy status; appendicular joint type level.

Appendix 3.4a Shoulder

Shoulder						
Predictor	**Sub-category**	**Total Count**	**Arthropathy Negative**		**Arthropathy Positive**	
			Number	**Percent**	**Number**	**Percent**
Species	Cheetah	14	13	92.9%	1	7.1%
	Lion	5	1	20.0%	4	80.0%
	Tiger	18	17	94.4%	1	5.6%
Gender	Female	12	12	100.0%	0	0.0%
	Male	25	19	76.0%	6	24.0%
Age Class	Young Adult	12	11	91.7%	1	8.3%
	Adult	8	6	75.0%	2	25.0%
	Senior	8	6	75.0%	2	25.0%
	Geriatric	9	8	88.9%	1	11.1%
Grand Total		**37**	**31**	**83.8%**	**6**	**16.2%**

Appendix 3.4b Elbow

Elbow

Predictor	Sub-category	Total Count	Arthropathy Negative		Arthropathy Positive	
			Number	Percent	Number	Percent
Species	Cheetah	21	21	100.0%	0	0.0%
	Lion	19	15	78.9%	4	21.1%
	Tiger	24	16	66.7%	8	33.3%
Gender	Female	22	19	86.4%	3	13.6%
	Male	42	33	78.6%	9	21.4%
Age Class	Young Adult	25	23	92.0%	2	8.0%
	Adult	12	10	83.3%	2	16.7%
	Senior	15	11	73.3%	4	26.7%
	Geriatric	12	8	66.7%	4	33.3%
Grand Total		**64**	**52**	**81.3%**	**12**	**18.8%**

Appendix 3.4c Carpus

Carpus

Predictor	Sub-category	Total Count	Arthropathy Negative		Arthropathy Positive	
			Number	Percent	Number	Percent
Species	Cheetah	28	26	92.9%	2	7.1%
	Lion	14	13	92.9%	1	7.1%
	Tiger	21	20	95.2%	1	4.8%
Gender	Female	20	20	100.0%	0	0.0%
	Male	43	39	90.7%	4	9.3%
Age Class	Young Adult	29	26	89.7%	3	10.3%
	Adult	9	8	88.9%	1	11.1%
	Senior	12	12	100.0%	0	0.0%
	Geriatric	13	13	100.0%	0	0.0%
Grand Total		**63**	**59**	**93.7%**	**4**	**6.3%**

Appendix 3.4d Coxofemoral joint

Coxofemoral joint						
Predictor	**Sub-category**	**Total Count**	**Arthropathy Negative**		**Arthropathy Positive**	
			Number	**Percent**	**Number**	**Percent**
Species	Cheetah	53	51	96.2%	2	3.8%
	Lion	20	20	100.0%	0	0.0%
	Tiger	38	27	71.1%	11	28.9%
Gender	Female	48	43	89.6%	5	10.4%
	Male	63	55	87.3%	8	12.9%
Age Class	Young Adult	46	42	91.3%	4	8.7%
	Adult	25	21	84.0%	4	16.0%
	Senior	23	19	82.6%	4	17.4%
	Geriatric	17	16	94.1%	1	5.9%
Grand Total		**111**	**98**	**88.3%**	**13**	**11.7%**

Appendix 3.4e Stifle

Stifle						
Predictor	**Sub-category**	**Total Count**	**Arthropathy Negative**		**Arthropathy Positive**	
			Number	**Percent**	**Number**	**Percent**
Species	Cheetah	31	26	83.9%	5	16.1%
	Lion	20	10	50.0%	10	50.0%
	Tiger	24	23	95.8%	1	4.2%
Gender	Female	28	22	78.6%	6	21.4%
	Male	47	37	78.7%	10	21.3%
Age Class	Young Adult	31	25	80.6%	6	19.4%
	Adult	12	10	83.3%	2	16.7%
	Senior	14	12	85.7%	2	14.3%
	Geriatric	18	12	66.7%	6	33.3%
Grand Total		**75**	**59**	**78.8%**	**16**	**21.3%**

Appendix 3.4f Tarsus

Tarsus

Predictor	Sub-category	Total Count	Arthropathy Negative		Arthropathy Positive	
			Number	Percent	Number	Percent
Species	Cheetah	22	20	90.9%	2	9.1%
	Lion	19	19	100.0%	0	0.0%
	Tiger	12	9	75.0%	3	25.0%
Gender	Female	23	21	91.3%	2	8.7%
	Male	30	27	90.0%	3	10.0%
Age Class	Young Adult	16	16	100.0%	0	0.0%
	Adult	10	7	70.0%	3	30.0%
	Senior	14	13	92.9%	1	7.1%
	Geriatric	13	12	92.3%	1	7.7%
Grand Total		**53**	**48**	**90.6**	**5**	**9.4%**

Appendix 3.5 a-c Significant predictors for arthropathies, appendicular joint type level. Predicted mean prevalence, standard error of the mean, and least significant differences of predictions (5%) level are presented.

Appendix 3.5a Shoulder: significant association with species

Species	Prediction[1]	s.e.[2]	Least significant differences of predictions (5% level)			
Cheetah	0.0714	0.0688	Cheetah	*		
Lion	0.8	0.1789	Lion	0.3895	*	
Tiger	0.0556	0.0539	Tiger	0.1776	0.3797	*
				Cheetah	Lion	Tiger

Appendix 3.5b Coxofemoral joint: significant association with species

Species	Prediction	s.e.	Least significant differences of predictions (5% level)			
Cheetah	0.0377	0.02617	Cheetah	*		
Lion	0.0001	0.00114	Lion	0.0519	*	
Tiger	0.2895	0.07357	Tiger	0.1548	0.1458	*
				Cheetah	Lion	Tiger

Appendix 3.5c Stifle: significant association with species

Species	Prediction	s.e.	Least significant differences of predictions (5% level)			
Cheetah	0.1613	0.06602	Cheetah	*		
Lion	0.5	0.11181	Lion	0.2588	*	
Tiger	0.0417	0.04042	Tiger	0.1543	0.237	*
				Cheetah	Lion	Tiger

[1] prediction denotes average predicted prevalence as a %

[2] s.e. denotes standard error of the mean

Appendix 3.6 P values for the modelling of associations between predictors and arthropathies of six appendicular joint types. Significant associations are highlighted in red.

Site	Age Class	Species	Gender	Age Species Interaction	Age Gender Interaction	Gender Species Inter-action
Shoulder	0.678	0.009	0.951	1	1	0.94
Elbow	0.33	0.74	0.56	0.896	0.789	0.915
Carpus	0.988	0.936	0.756	1	1	1
Coxofemoral	0.568	0.013	0.887	1	0.674	0.954
Stifle	0.549	0.004	0.948	1	0.984	0.825
Tarsus	0.413	0.458	0.872	1	0.998	0.947

Appendix 3.7 a-c Frequency of appendicular joint arthropathies for cheetah, lion and tiger, at the level of 'animal within age class'. Key findings are highlighted in red.

Appendix 3.7a Cheetah

		CHEETAH		
Age Class	**Appendicular joint**	**Total Number**	**Number arthropathy positive**	**Percent arthropathy positive**
Young Adult	Shoulder	10	2	20.0%
	Elbow	23	0	0.0%
	Carpus	30	2	6.7%
	Coxofemoral	52	1	1.9%
	Stifle	25	3	12.0%
	Tarsus	12	0	0.0%
Young Adult Total		**152**	**8**	**5.3%**
Adult	Shoulder	5	0	0.0%
	Elbow	2	0	0.0%
	Carpus	3	0	0.0%
	Coxofemoral	18	2	11.1%
	Stifle	9	1	11.1%
	Tarsus	10	2	20.0%
Adult Total		**47**	**5**	**10.6%**
Senior	Shoulder	3	0	0.0%
	Elbow	8	0	0.0%
	Carpus	10	0	0.0%
	Coxofemoral	22	0	0.0%
	Stifle	11	0	0.0%
	Tarsus	13	0	0.0%

Senior Total		**67**	**0**	**0%**
Geriatric	Shoulder	6	0	0.0%
	Elbow	4	0	0.0%
	Carpus	7	0	0.0%
	Coxofemoral	14	0	0.0%
	Stifle	7	0	0.0%
	Tarsus	4	0	0.0%
Geriatric Total		**42**	**0**	**0%**
Grand Total		**308**	**13**	**4.2%**

Appendix 3.7b Lion

Age Class	Appendicular joint	LION		
		Total Number	Number arthropathy positive	Percent arthropathy positive
Young Adult	Shoulder	0	N/A	N/A
	Elbow	11	1	9.1%
	Carpus	6	1	16.7%
	Coxofemoral	11	0	0.0%
	Stifle	11	2	18.2%
	Tarsus	9	0	0.0%
Young Adult Total		**48**	**4**	**8.3%**
Adult	Shoulder	2	1	50.0%
	Elbow	7	2	28.6%
	Carpus	3	0	0.0%
	Coxofemoral	11	0	0.0%
	Stifle	2	1	50.0%
	Tarsus	3	0	0.0%
Adult Total		**28**	**4**	**14.3%**
Senior	Shoulder	3	2	66.7%
	Elbow	6	2	33.3%
	Carpus	6	0	0.0%
	Coxofemoral	8	0	0.0%
	Stifle	8	4	50.0%
	Tarsus	7	0	0.0%
Senior Total		[38] **38**	**8**	**21.1%**
Geriatric	Shoulder	3	1	33.3%

	Elbow	8	1	12.5%
	Carpus	6	0	0.0%
	Coxofemoral	8	0	0.0%
	Stifle	14	7	50.0%
	Tarsus	13	0	0.0%
Geriatric Total		**52**	**9**	**17.3%**
Grand Total		**166**	**25**	**15.1%**

Appendix 3.7c Tiger

Age Class	Appendicular joint	TIGER		
		Total Number	Number arthropathy positive	Percent arthropathy positive
Young Adult	Shoulder	12	0	0.0%
	Elbow	11	1	9.1%
	Carpus	10	0	0.0%
	Coxofemoral	27	4	14.8%
	Stifle	13	0	0.0%
	Tarsus	7	0	0.0%
	Total	**80**	**5**	**6.3%**
Adult	Shoulder	8	2	25.0%
	Elbow	9	1	11.1%
	Carpus	8	1	12.5%
	Coxofemoral	20	3	15.0%
	Stifle	9	0	0.0%
	Tarsus	3	1	33.3%
	Total	**57**	**8**	**14.0%**
Senior	Shoulder	6	0	0.0%
	Elbow	13	4	30.8%
	Carpus	7	0	0.0%
	Coxofemoral	16	5	31.3%
	Stifle	7	0	0.0%
	Tarsus	5	1	20.0%
	Total	**54**	**10**	**18.5%**
Geriatric	Shoulder	5	0	0.0%
	Elbow	5	4	80.0%

	Carpus	8	0	0.0%
	Coxofemoral	12	1	8.3%
	Stifle	9	1	11.1%
	Tarsus	5	2	40.0%
Geriatric Total		**44**	**8**	**18.2%**
Grand Total		**235**	**31**	**13.2%**

Appendix 3.8 a–c Tabulations of average maximum severity for six appendicular joint types, for three species, age classes pooled, arthropathy-positive joints only.

Appendix 3.8a Cheetah

CHEETAH

Appendicular joint	Total Number	Average maximum severity	Average maximum age class
Shoulder	2	2.5	1
Elbow	0	N/A	N/A
Carpus	2	2	1
Coxofemoral	3	1.7	1.7
Stifle	3	1.7	1.3
Tarsus	2	2.5	2

Appendix 3.8b Lion

LION

Appendicular joint	Total Number	Average maximum severity	Average maximum age class
Shoulder	3	1.7	3.0
Elbow	6	1.7	2.5
Carpus	1	1.0	1.0
Coxofemoral	0	N/A	N/A
Stifle	12	1.2	3.2
Tarsus	0	N/A	N/A

Appendix 3.8c Tiger

TIGER

Segment	Total Number	Average maximum severity	Average maximum age class
Shoulder	2	3	2
Elbow	10	1.7	3.1
Carpus	1	1	2
Coxofemoral	10	1.8	2.3
Stifle	1	1	4
Tarsus	4	2.25	3.25

Appendix 3.9 a-d Unilateral versus bilateral distribution of appendicular arthropathies.

Appendix 3.9a Unilateral versus bilateral distribution of appendicular arthropathies according to JOINT TYPE.

Joint Type	Total number of Joint Type	Number with unilateral distribution	Percentage with unilateral distribution	Number with bilateral distribution	Percentage with bilateral distribution
Shoulder	5	3	60%	2	40%
Elbow	8	4	50%	4	50%
Carpus	3	3	100%	0	0 %
Coxofemoral	12	9	75%	3	25 %
Stifle	10	6	60%	4	40%
Tarsus	5	4	80%	1	20%
Grand Total	43	29	67.4 %	14	32.6%

Appendix 3.9b Unilateral versus bilateral distribution of appendicular arthropathies according to AGE CLASS.

Age Class	Total number of Joint Type	Number with unilateral distribution	Percentage with unilateral distribution	Number with bilateral distribution	Percentage with bilateral distribution
Young Adult	11	9	81.8%	2	18.2%
Adult	12	9	75%	3	25 %
Senior	11	6	54.5%	5	45.5%
Geriatric	9	5	55.5%	4	44.5%
Grand Total	43	29	67.4%	14	32.6%

Appendix 3.9c Unilateral versus bilateral distribution of appendicular arthropathies according to SPECIES.

Species	Total number of Joint Type	Number with unilateral distribution	Percentage with unilateral distribution	Number with bilateral distribution	Percentage with bilateral distribution
Cheetah	9	7	77.8%	2	22.2%
Lion	15	9	60%	6	40%
Tiger	19	13	68.4%	6	31.6%
Grand Total	43	29	67.4%	14	32.6%

Appendix 3.9d Unilateral versus bilateral distribution of appendicular arthropathies, for joint type within age class and species.

Species	Age Class	Joint Type	Total number of Joint Type	Unilateral distribution		Bilateral distribution	
				Number	Percent	Number	Percent
Cheetah	**1**	Shoulder	1	0	0.0%	1	100%
		Carpus	2	2	100%	0	0.0%
		Coxofemoral	1	1	100%	0	0.0%
		Stifle	2	2	100%	0	0.0%
		Total	**6**	**5**	**83.3%**	**1**	**16.7%**
	2	Coxofemoral	1	0	0.0%	1	100 %
		Tarsus	2	2	100%	0	0.0%
		Total	**3**	**2**	**66.7%**	**1**	**33.3%**
Cheetah Total			**9**	**7**	**77.8%**	**2**	**22.2%**
Lion	**1**	Elbow	1	1	100.0%	0	0.0%
		Stifle	1	1	100.0%	0	0.0%
		Total	**2**	**2**	**100.0%**	**0**	**0.0%**
	2	Shoulder	1	1	100.0%	0	0.0%
		Elbow	1	0	0.0%	1	100%
		Stifle	1	1	100.0%	0	0.0%
		Total	**3**	**2**	**66.7%**	**1**	**33.3%**
	3	Shoulder	1	1	100.0%	0	0.0%
		Elbow	1	0	0.0%	1	100%
		Stifle	2	0	0.0%	2	100%
		Total	**4**	**1**	**25.0%**	**3**	**75.0%**
	4	Shoulder	1	1	100%	0	0.0%%
		Elbow	1	1	100%	0	0.0%

		Stifle	4	2	50.0%	2	50.0%
		Total	**6**	**4**	**66.7%**	**2**	**33.3%**
Lion Total			**15**	**9**	**60.0%**	**6**	**40.0%**
Tiger	**1**	Elbow	1	1	100%	0	0.0%
		Coxofemoral	2	1	50.0%	1	50.0%
		Total	**3**	**2**	**66.7%**	**1**	**33.3%**
	2	Shoulder	1	0	0.0%	1	100%
		Carpus	1	1	100%	0	0.0%
		Coxofemoral	3	3	100%	0	0.0%
		Tarsus	1	1	100%	0	0.0%
		Total	**6**	**5**	**83.3%**	**1**	**16.7%**
	3	Elbow	2	1	50.0%	1	50.0%
		Coxofemoral	4	3	75.0%	1	25.0%
		Tarsus	1	1	100%	0	0.0%
		Total	**7**	**5**	**71.4%**	**2**	**28.6%**
	4	Elbow	1	0	0.0%	1	100%
		Coxofemoral	1	1	100%	0	0.0%
		Tarsus	1	0	0.0%	1	100%
		Total	**3**	**1**	**33.3%**	**2**	**66.7%**
Tiger Total			**19**	**13**	**68.4%**	**6**	**31.6%**
Grand Total			**43**	**29**	**67.4%**	**14**	**32.6%**

Appendix 3.10 a-b Nature of appendicular arthropathies: distribution of data set.

Appendix 3.10a Age class within species.

Species	Age Class At Xray	Number joints per age class	Age class percentage per species
Cheetah	1	9	64.3%
	2	5	35.7%
Cheetah Total		**14**	**19.4%**
Lion	1	4	16.0%
	2	4	16.0%
	3	8	32.0%
	4	9	36.0%
Lion Total		**25**	**34.7%**
Tiger	1	5	15.2%
	2	9	27.3%
	3	11	33.3%
	4	8	24.2%
Tiger Total		**33**	**45.8%**
Grand Total		**72**	**100%**

Appendix 3.10b Appendicular joint type within species.

	Total appendicular joints		Appendicular joints: Cheetah		Appendicular joints: Lion		Appendicular joints: Tiger	
Appendicular joint type	**Number**	**Percent**	**Number**	**Percent**	**Number**	**Percent**	**Number**	**Percent**
Shoulder	9	12.5%	3	21.4%	4	16.0%	2	6.1%
Elbow	16	22.2%	0	0.0%	6	24.0%	10	30.3%
Carpus	4	5.6%	2	14.3%	1	4.0%	1	3.0%
Coxofemoral	17	23.6%	3	21.4%	0	0.0%	14	42.4%
Stifle	18	25.0%	3	21.4%	14	56.0%	1	3.0%
Tarsus	8	11.1%	3	21.4%	0	0.0%	5	15.2%
Grand Total	**72**	**100%**	**14**	**100%**	**25**	**100%**	**33**	**100%**

Appendix 3.11 a-c Details of the nature of appendicular arthropathies. M; Male, F; Female, CT; computed tomography, OC: osteochondrosis, OCD; osteochondritis dissecans.

Appendix 3.11a Cheetah

Case No.	Gen-der	Age Class	Site	Nature	Details	Follow-up Radiographs	Comments
1	M	2	Tarsus right	Traumatic	Intraarticular fracture	No	Records for following 2 years show no further radiographs. No further information available.
2	M	2	Coxofemoral bilateral	Degenerative: no inciting cause identified	Enthesophytosis (Morgan lines) only	Yes	Repeatable but nonprogressive changes seen six months later. No further radiographs for following 6 years.
3	M	1	Carpus left	Degenerative: postdevelopmental, postsurgical correction	Unilateral OA presumed secondary to prior distal ulnar metaphyseal osteochondrosis. Developmental disease was bilateral but secondary OA unilateral.	No	Degenerative changes first noted 2 years postsurgical correction. No further radiographs available for this joint.
4	M	1	Stifle left	Traumatic	Intraarticular fracture	Yes	2 months after diagnosis: no degenerative changes seen, fracture resolving.
5	M	1	Stifle left	Degenerative: postdevelopmental, postsurgical correction	Medially luxating patella with secondary femoropatellar OA, intraarticular osseous body. Developed severe stifle OA secondary to patella luxation and corrective surgery.	No	Transferred 6 months later - lost to follow up.

Case No.	Gen-der	Age Class	Site	Nature	Details	Follow-up Radiographs	Comments
6	F	1	Stifle right	Degenerative: postsepsis	OA secondary to disseminated fungal disease.	No	Died 1 week later.
7	M	1	Shoulder right	Septic	OA associated with multifocal osteomyelitis/septic polyarthropathy.	Yes	4 month follow up radiographs showed resolution of pathology. No degenerative changes noted.
8	M	1	Coxofemoral right	Septic	OA associated with multifocal osteomyelitis/septic polyarthropathy.	No	Unknown: there are no further clinical records available for this case.
9a	M	1	Shoulder left	Developmental	OC/OCD	Yes	4 month follow up radiographs showed progression to degenerative changes -see 9b.
9b	M	1	Shoulder left	Degenerative: postdevelopmental	OA severe	No	Unknown: there are no further clinical records available for this case.
10	M	2	Stifle left	Degenerative: no inciting cause identified	Mild femoropatellar OA	Yes	3.5 years later-radiographic changes not repeatable.
11a	F	1	Tarsus left	Developmental	Left tibiotarsal OC/OCD, avulsion fracture left medial malleolus	Yes	3 months post diagnosis/2 weeks post corrective surgery
11b	F	1	Tarsus left	Degenerative: postdevelopmental	Moderate osteophytosis secondary to OC/OCD	No	Recent case
12	M	1	Carpus left	Traumatic	Malunion intraarticular fracture, accessory carpal bone	No	Lost to follow up-transferred 2 years post injury.

Appendix 3.11b Lion

Case No.	Gen-der	Age Class	Site	Nature	Details	Follow up Radiographs	Comments
1	M	4	Elbow left	Degenerative: no inciting cause identified	OA: mild osteophytosis	No	Imaging performed at end of life.
2a	M	3	Shoulder right	Degenerative: no inciting cause identified	OA: mild osteophytosis	Yes	Imaged 4 months later-see case2b.
2b	M	4	Shoulder right	Degenerative: no inciting cause identified	OA: mild osteophytosis	No	Imaging performed at end of life.
3	M	4	Stifle right	Degenerative: no inciting cause identified	Mild osteophytic femoropatellar OA	No	Imaging performed at end of life.
4	M	4	Stifle left	Degenerative: no inciting cause identified	Mild osteophytic femoropatellar OA	No	Imaging performed at end of life.
5	F	1	Stifle left	Degenerative: post traumatic	Intra-articular chip fracture and enthesophytosis	No	Penetrating wound to site 3 month prior to imaging. No further imaging of joint.
6	F	4	Stifle left	Degenerative: no inciting cause identified	Mild osteophytic femoropatellar OA	No	Died 7 months later with no further imaging.
7	M	1	Stifle left	Degenerative: no inciting cause identified	Mild joint-associated soft tissue mineralisation and joint effusion	No	Recent case.
8	F	4	Stifle left	Degenerative: no inciting cause identified	OA: mild osteophytosis and dystrophic soft tissue mineralisation	Not available	No further information and died 2 years later.

Case No.	Gen-der	Age Class	Site	Nature	Details	Follow up Radiographs	Comments
9	M	1	Carpus right	Unclear	Two intraarticular osseous bodies	No	No further imaging of this joint up to 8 years later.
10	M	2	Elbow left	Degenerative: no inciting cause identified	DJD: enthesophytosis only	No	Lost to follow up.
11	M	2	Elbow right	Degenerative: no inciting cause identified	OA moderate	No	Lost to follow up.
12	M	2	Shoulder right	Degenerative: post developmental	OA severe: secondary to OC/OCD of caudal humeral head diagnosed 8 years prior	No	Lost to follow up.
13	F	1	Elbow right	Traumatic	Intraarticular fracture	Yes	Surgical fixation. Follow up radiographs for next 6 years found no degenerative changes.
14	M	2	Stifle right	Degenerative: post traumatic?	Radiographic changes consistent with moderate OA . Partial tear of caudal cruciate ligament insertion confirmed on arthroscopy 12 months prior	No	Necropsy examination 9 months later confirmed moderate-severe OA
15a	F	3	Stifle left	Degenerative?: no inciting cause identified	OA mild with synovitis	Yes	Imaged 3 years later-see 15b
15b	F	4	Stifle left	Degenerative: post developmental or synovial osteochondroma?	OA moderate with non meniscal ossicle osseous body; synovial osteochondroma? OCD fragment?	Yes	Not available for inclusion.

Case No.	Gen-der	Age Class	Site	Nature	Details	Follow up Radiographs	Comments
16a	F	3	Stifle right	Developmental or synovial osteochondroma	Non-meniscal intraarticular osseous body; OCD? Or synovial osteochondroma?	Yes	Imaged 3 years later-see 16b
16b	F	4	Stifle right	Degenerative: post developmental	Mild OA secondary to OCD lesion identified in femoral condyle	Yes	Not available for inclusion.
17	M	4	Stifle right	Degenerative: no inciting cause identified	mild osteophytic femoropatellar OA	No	Died 12 months later with no further imaging.
18	M	3	Elbow left	Degenerative: no inciting cause identified	OA severe with osteo- and enthesophytosis and intra-articular osseous body of unknown origin	No	Imaging performed at end of life.
19	M	3	Elbow right	Degenerative: no inciting cause identified	OA moderate with osteo-and enthesophytosis	No	Imaging performed at end of life.
20	M	3	Shoulder right	Degenerative: no inciting cause identified	OA mild with osteophytes rim of caudal glenoid cavity	No	Imaging performed at end of life.
21	M	3	Stifle left	Degenerative: no inciting cause identified	mild osteophytic femoropatellar OA	No	Imaging performed at end of life.
22	M	3	Stifle right	Degenerative: no inciting cause identified	mild osteophytic femoropatellar OA	No	Imaging performed at end of life.

Appendix 3.11c Tiger

Case No.	Gen-der	Age Class	Site	Nature	Details	Follow up Radiographs	Comments
1a	M	1	Coxofemoral right	Degenerative: postdevelopmental (consistent with hip dysplasia)	OA mild: osteophyte cranial acetabular rim	Yes	Imaged in following age class. See 1b.
1b	M	2	Coxofemoral right	Degenerative: postdevelopmental (consistent with hip dysplasia)	OA mild: osteophyte cranial acetabular rim	No	Died 4 years later with no further imaging of this site.
2	M	2	Shoulder left	Degenerative: no inciting cause identified	OA severe	No	Lost to follow up.
3	M	2	Shoulder right	Degenerative: no inciting cause identified	OA severe	No	Lost to follow up.
4	M	2	Elbow Right	Unclear	Changes confined to intraarticular osseous body; origin unclear. Developmental? Synovial osteochondroma? Other? no evidence of degenerative changes.	No	Current case.
5	M	2	Tarsus left	Degenerative: no inciting cause identified	OA moderate with osteo- and enthesophytosis	No	Current case.
6	M	3	Coxofemoral left	Degenerative: no inciting cause identified	Enthesophytosis (Morgan line) only	No	Current case.

Case No.	Gen-der	Age Class	Site	Nature	Details	Follow up Radiographs	Comments
7	M	3	Elbow left	Degenerative: no inciting cause identified	OA mild	No	Died 4 months later with no further imaging of this site.
8	M	4	Elbow left	Degenerative: no inciting cause identified	OA severe: osteo- and enthesophytosis	No	Imaging performed at end of life.
9	M	4	Elbow left	Degenerative: no inciting cause identified	OA moderate with osteo- and enthesophytosis	No	Died 14 months later with no further imaging of this site.
10	M	4	Elbow Right	Degenerative: no inciting cause identified	OA moderate with osteo- and enthesophytosis	No	Died 14 months later with no further imaging of this site.
11	F	1	Coxofemoral left	Degenerative: postdevelopmental (hip dysplasia)	OA mild: osteophytosis secondary to presumed hip dysplasia	No	Current case.
12	F	1	Coxofemoral right	Degenerative: postdevelopmental (hip dysplasia)	OA mild: osteophytosis secondary to presumed hip dysplasia	No	Current case.
13	M	1	Coxofemoral right	Developmental	Developmental malformation most likely secondary to slipped capitus femoris or less likely avascular necrosis of femoral head. Absence of a formed acetabulum.	No	9 years follow up with no further imaging.
14a	F	2	Coxofemoral left	Traumatic	Intraarticular fracture of acetabulum	Yes	Imaged 6 weeks post injury. See 14b.
14b	F	2	Coxofemoral left	Degenerative: post traumatic	OA: mild osteophytosis, severe enthesophytosis	Yes	Imaged 2 years post injury. See 14c.

Case No.	Gen-der	Age Class	Site	Nature	Details	Follow up Radiographs	Comments
14c	F	3	Coxofemoral left	Degenerative: post traumatic	OA: moderate osteophytosis with malunion of fracture site	Yes	Imaged 5 years post injury. See 14d.
14d	F	4	Coxofemoral left	Degenerative: post traumatic	OA: moderate osteophytosis with malunion of fracture site	Yes	Imaged 7 and 8 years post injury. No progression of radiographic changes. Delayed union of acetabular fracture with moderate OA.
15	F	4	Elbow left	Degenerative: no inciting cause identified	DJD: joint-associated dystrophic mineralisation	No	Recent case.
16	F	4	Tarsus left	Degenerative: no inciting cause identified	DJD: severe enthesophytosis	No	Died 18 months later with no further imaging of this site.
17	F	4	Tarsus right	Degenerative: no inciting cause identified	OA severe: osteo- and enthesophytosis	No	Died 7 years later with no further imaging of this site.
18	M	2	Coxofemoral left	Degenerative: postdevelopmental suspected (hip dysplasia?)	OA moderate: osteophytosis cranial and caudal acetabular rims	No	Died 18 months later with no further imaging of this site.
19	M	2	Carpus right	Degenerative: no inciting cause identified	OA mild	Yes	Mild OA on CT 14 years later.
20	M	3	Elbow left	Degenerative: no inciting cause identified	OA severe	Yes	Severe OA on CT 5 years later.
21a	M	3	Tarsus left	Traumatic	Intraarticular fracture with numerous osseous bodies detected, and	Yes	Imaged 12 months later. See 21b.

Case No.	Gen-der	Age Class	Site	Nature	Details	Follow up Radiographs	Comments
					associated bone defect distal tibia		
21b	M	3	Tarsus left	Degenerative: post traumatic	OA mild	Yes	Severe OA on CT 5 years later.
22	M	3	Coxofemoral left	Degenerative: postdevelopmental suspected (hip dysplasia?)	OA mild: remodeling cranial acetabular rim	No	Died 3 years later and no further images available for this site.
23	F	3	Coxofemoral left	Degenerative: no inciting cause identified	OA severe	No	Recent case.
24	F	3	Coxofemoral right	Degenerative: no inciting cause identified	OA severe	No	Recent case.
25	F	3	Elbow left	Degenerative: post developmental suspected?	DJD: moderate enthesophytosis	No	Recent case.
26	F	3	Elbow right	Degenerative: no inciting cause identified	OA mild: osteophytosis cranial margin distal humerus	No	Recent case.
27	M	1	Elbow right	Developmental	Suspect OC/OCD: small area of lucency lateral humeral condyle on radiography	Yes	2 years later: radiographic changes not repeatable.
28	M	4	Stifle left	Degenerative: no inciting cause identified	Mild osteophytic femoropatellar OA	No	Imaging performed at end of life.

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Chapter 4

A retrospective radiological study of arthropathies of captive lions, tigers, and cheetahs

Part III: The Total Skeleton and a comparison of arthropathies of the axial and appendicular skeletons

4.1 Introduction

In Chapters 2 and 3, the prevalence, distribution and features of arthropathies of the axial and appendicular skeletons of captive cheetahs, lions and tigers have been described. In this Chapter, these two skeletal levels have been combined, and the skeleton is considered as a single unit (referred to as the total skeleton). This has enabled an assessment of the prevalence of joint disease across the skeleton, and further investigation of factors associated with radiographic detection of joint disease.

The aims of this broader investigation are therefore to provide zoo clinicians with the likelihood of joint disease detection in the large cat patient, and, through combining information from both the axial and appendicular skeletons, maximise the opportunity to detect significant associations between potential risk factors and joint disease. This chapter concludes with a comparison of findings from the axial and appendicular skeletons as reported in Chapters 2 and 3 respectively. This discussion will focus on key similarities and differences, as well as an assessment of the respective contributions of the two skeletal levels to the overall picture of joint disease, in particular degenerative joint disease (DJD), in captive lions, tigers and cheetahs.

4.2 Materials and Methods

Analyses of arthropathies at the level of the total skeleton included arthropathy prevalence, and predictors of arthropathy status. All analyses were performed after radiographic scoring of features of arthropathies for both the axial and appendicular skeleton.

4.2.1 Data acquisition and age class classification

Data acquisition is as previously described in Chapters 2 and 3, resulting in the identification of 702 radiographic studies from 305 animals. Studies were included if the animal was six months or older at the time of imaging, and included one or more images of diagnostic quality, that captured all musculoskeletal anatomy of any of the following: one or more intervertebral joints, the sacroiliac joint, appendicular joint types shoulder, elbow, carpus, coxofemoral joint, stifle or tarsus. A total of 564 studies of 254 animals, containing 2286 images met the inclusion criteria for an investigation of arthropathies of the total skeleton. Of these studies, 281 studies captured the axial skeleton only, 95 studies captured the appendicular skeleton only, and 188 studies captured joints from both the axial and appendicular skeletons. Animals were then categorised into four age classes: young adult, adult, senior and geriatric, as described in Chapter 2.

4.2.2 Radiological scoring

An arthropathy score of 0 (negative) or 1 (positive) for the total skeleton was then ascribed to every study. This score was based on the arthropathy score(s) that the study achieved for its axial and/or appendicular skeletal level components, with a positive score (1) assigned if either or both the axial and appendicular skeletal levels scored a positive arthropathy status. Therefore, a positive arthropathy score at the total skeletal level was consistent with arthropathy detected at one or more joints ((intervertebral, sacroiliac, shoulder, elbow, carpus, coxofemoral, stifle, tarsus). As described in Chapters 2 and 3, for every radiographic study, animal identification data, and specific data related to the imaging study (date, institution, animal age at imaging, number of readable images, input from second reviewer) was recorded in a spreadsheet [Microsoft ® Excel 2016) database.

4.2.3 Data preparation for analysis

Many animals underwent imaging that captured one or more joints, on multiple occasions, and across multiple age classes. As with Chapters 2 and 3, it was therefore necessary to identify and remove repeat observations in preparation for statistical analysis, such that the final data set for the determination of arthropathy prevalence, and modeling of predictors was restricted to a maximum of one study per animal per age class. For animals with multiple eligible studies within a single age class, the protocol to determine which study was to be included for that age class is as follows. If all studies for an animal within an age class were arthropathy positive at the level of total skeleton, then the earliest study was included. If all studies were negative, then the most recent study was included. For those animals where the arthropathy status for the total skeleton transitioned from negative to positive within an age class, the earliest study to record a positive arthropathy status was included. The outcome was a final data set where each 'study' represented an 'animal within an age class', with the 'total skeleton' arthropathy score for that study attached.

4.2.4 Statistical analysis: Inferential and descriptive statistics

Inferential statistical analysis was performed as described in Chapters 2 and 3. Logistic regression via a generalised linear mixed model with an underlying binomial distribution (GenStat, Version17, VSNi) was used to explore the association between the arthropathy status (0,1) of the total skeleton, and the following predictors: species, gender, age class, and their interactions. All modelling was performed at an 'animal within an age class' level, with animal identification included as a random effect. In addition, due to the high number of cheetahs in the study, and their dispersal between open-range and urban zoos, the effect of enclosure size on the prevalence of cheetah arthropathies was also investigated. Due to the weighting of cheetah numbers for one open-range institution (Institution A), this was modelled at two levels: urban versus open-range, and Institution A versus all other zoos. Interactions between cheetah enclosure size, age, and gender were also modelled.

Modelling was based on a binary output for arthropathy status (0,1). All predictors were categorical, and either nominal with several categories (species), nominal and dichotomous

(gender, cheetah enclosure size) or ordinal (age class). The level of statistical significance for association between arthropathy status and potential predictors was set at $P<0.05$. Due to small data set size for some analyses, a more liberal P-value range of ≥ 0.05 and <0.1 was considered trending to significance. Predicted means, with associated standard errors, were generated for all significant associations. Where indicated, least significant differences (LSDs), either manually calculated or computer-software generated (GenStat, Version17, VSNi), were used to perform pairwise comparisons of predicted means for subcategories within significant predictors. Descriptive statistics was restricted to determination of the prevalence of arthropathies of the total skeleton, and were conducted according to age class.

4.3 Results

4.3.1 Study Population

A total of 353 radiographic studies of 'animals within an age class', representing 254 individual animals, were analysed for the prevalence of arthropathy at the total skeleton level (Figure 4.1). One hundred and four of 353 (29.5%) of studies were positive for radiographically detectable arthropathy at one or more appendicular joints or axial segments. The frequency distribution of modelled predictors against arthropathy status is shown (Appendix 4.1).

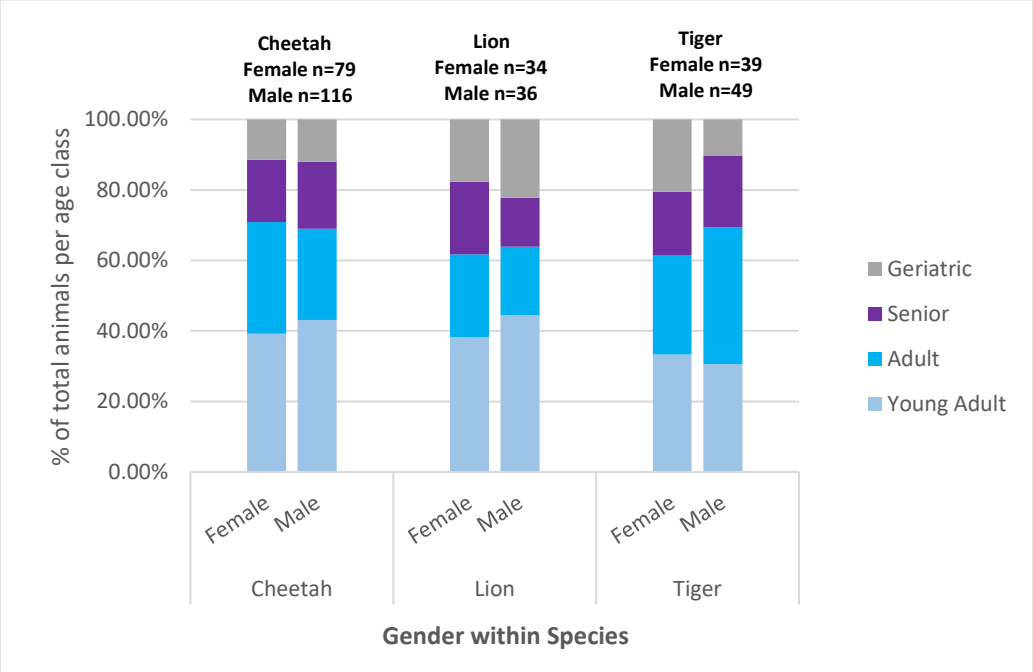

Figure 4.1 Distribution of 'animal within an age class' study population for analysis of arthropathies of the total skeleton. n= number of individuals, gender within species

4.3.2 Modelling for predictors of arthropathy status

The most significant predictor for the detection of arthropathies in the total skeleton was increasing age (P<0.001), with the predicted prevalence of arthropathies 7%, 16.3%, 17.7% and 77.4% for young adult through to geriatric age classes respectively. Pairwise comparisons showed that the prevalence of arthropathies in geriatric animals were significantly higher, and young adults significantly lower, than all other age classes, with no difference between the adult and

senior age classes. There was also a significant difference between species (P=0.027), with the mean predicted prevalence for cheetahs, lions and tigers 17.3%, 30% and 33.8% respectively. Pairwise comparisons showed that cheetahs were predicted to have a significantly lower prevalence of arthropathy than either the tiger or lion, with no difference between the two latter species. An age-species interaction was also seen, with LSD calculations showing that tigers were predicted to have significantly more arthropathies than lions in the senior age class, and cheetahs in the geriatric age class (Figure 4.2).

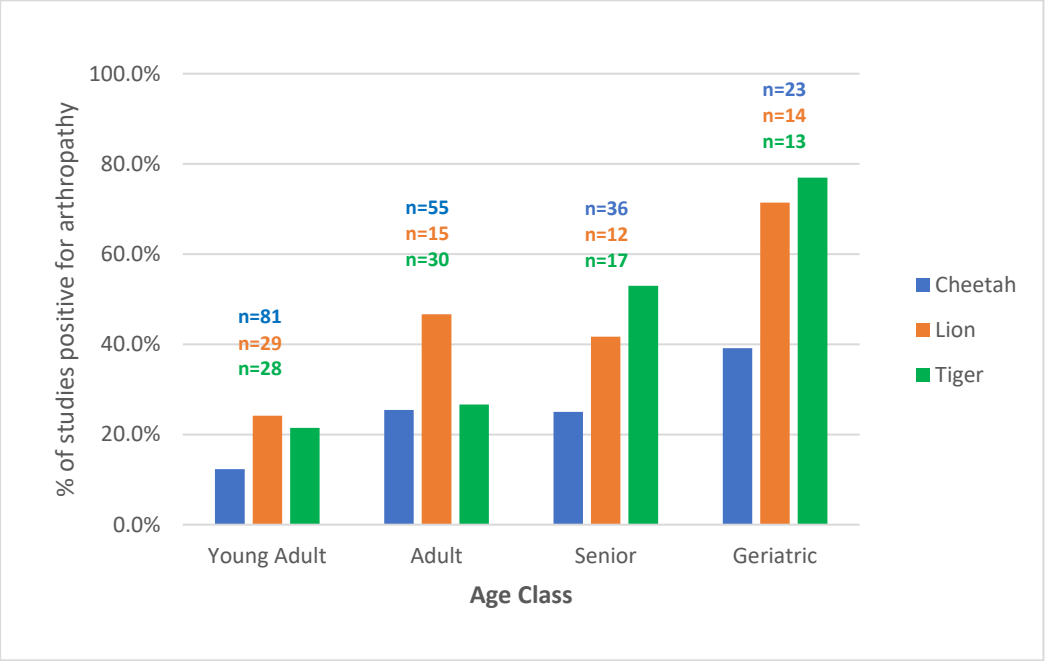

Figure 4.2 Distribution of the prevalence of arthropathies of the total skeleton for the cheetah, lion and tiger, as a function of age. n= number of cheetahs/lions/tigers in an age class

For the cheetah data subset, a significant association between enclosure size and arthropathy status was detected for both urban versus open-range zoos, and Institution A versus all other zoos. Cheetahs held in open-range zoos were significantly less likely to develop arthropathies than those held in urban zoos (P=0.016), with predicted mean prevalence of 7% versus 24.2% respectively. Cheetahs held at Institution A had a predicted prevalence of arthropathy of 5.9% compared with 17% for all other institutions combined (P=0.041). In addition, a significant interaction with both age class (P=0.046) and gender (P=0.039) was seen when Institution A was

modelled against all other zoos. Both female cheetahs, and cheetahs of senior and geriatric age classes, held at Institution A were predicted to have significantly lower arthropathy prevalence than their counterparts held at other institutions.

For all significant associations, the predicted means and standard errors for the differences, used for manual calculations of pairwise (LSD) calculations where indicated, are reported in Appendix 4.2. P values from all modelling can be found in Appendix 4.3.

4.4 Discussion

This study, of radiographically detectable joint disease across the entire skeleton of captive cheetahs, lions and tigers, is the first to report not only the prevalence of joint disease, but the relative contribution of DJD within the spectrum of all arthropathies as identified by radiography. Not only was joint disease found commonly in these animals, it was overwhelmingly more likely to be degenerative in nature, clarifying the relevance of DJD within the scope of possible joint diseases for these species. By combining axial and appendicular skeletal data, this investigation provided an enhanced ability to detect significant associations between predictors and arthropathy status that may have remained elusive in the smaller data sets. As a result, the likelihood of joint disease detection was found to be influenced by several key factors, consolidating findings from earlier chapters.

4.4.1 Factors affecting arthropathies of the total skeleton

Increasing age was the most significant factor affecting the likelihood of radiographic joint disease in these animals. In this regard, captive large cats are no different to their domestic counterparts.[1-7] The magnitude of the strength of association indicated the clinical importance of this finding, and consequently any further investigation of DJD in these three species of large cats must firstly account for the impact of increasing age. Critically, DJD in the captive-held cheetah, lion and tiger was most apparent in those extended years beyond what is usually reported for their free-ranging conspecifics,[8] posing the question as to whether this disease is an unavoidable consequence of the improved health and longevity of animals under human care. The prevalence of joint disease was also significantly different between the species, with the cheetah, yet again, significantly less likely to demonstrate radiographic joint disease, informing the recommendation that for future investigations of joint disease, large cat species be evaluated independently. Not only was a species difference identified, but it was found to be most apparent in the older age classes. Two key conclusions stemmed from this finding. Firstly, from a practical perspective, as the geriatric tiger was identified as most at risk of DJD, increased monitoring of ageing tigers is indicated. Secondly, the effect of age on joint disease in these species was shown to be more complex than a simplistic linear relationship. There was no universal or uniform

impact of age that extended to all three species, with the relative sparing of older cheetahs highlighting once again the potential contributions of size, subtle musculoskeletal differences, and as yet unidentified species -specific risk factors, to the occurrence of joint disease in the cheetah, lion and tiger.

Of particular significance was the association between enclosure size and arthropathies in captive-held cheetahs. By accommodating all joints in the skeleton, this study provided further evidence that larger enclosure sizes do exert a protective effect, with cheetahs held in urban zoos significantly more likely to have radiographically detectable joint disease. In addition, by comparing cheetahs from one particular open-range zoo (Institution A) against all others, further detail regarding potential impacts of enclosure size on joint disease in cheetahs was identified. Noting that Institution A accounted for 68% of open-range cheetahs, and 47% of all cheetahs in the study, the pattern of joint disease found in this cohort presented a very different picture to that seen elsewhere. The prevalence of joint disease for female cheetahs from Institution A was significantly less than for all other female cheetahs in this study. The reasons for this finding are unclear but may in part relate to differing management practices. This institution is a key captive cheetah breeding facility and consequently houses not only a significantly higher number of females at any one time, but, as part of breeding operations, females are rotated both between different enclosures on site, and transferred between institutions. Thus, their length of stay at this institution varied dramatically, and may have contributed some bias to this result. However, the finding of a relatively stable prevalence of joint disease between age classes for cheetahs held at Institution A is significantly different to the rise in joint disease between senior and geriatric age classes seen in cheetahs from other zoos. This result may indeed reflect a protective effect provided by the generally larger enclosure sizes seen at this open-range institution, and the subsequent increased opportunity to express more natural behaviours. On the basis of these findings, and particularly when combined with those from the similar investigation for the axial skeleton, any decision to exhibit cheetahs in smaller enclosures invites serious reappraisal.

4.4.2 A comparison of joint disease of the axial versus the appendicular skeleton

The similar prevalence of axial versus appendicular arthropathies reported in the earlier chapters indicates that joint disease is as likely to be detected in the axial as the appendicular skeleton. However, these results should be considered an approximation only, as complete radiographic coverage of all axial segments and appendicular joints was unavailable for the vast majority of study subjects. In addition, whilst many images of the axial skeleton, and therefore of axial DJD, were captured incidentally, with few exceptions appendicular joints were imaged as part of a diagnostic investigation for musculoskeletal issues. Thus, due to the retrospective and opportunistic nature of data collection, bias at multiple levels may have been inadvertently introduced into the reporting of prevalence for the two skeletal levels.

Both skeletal levels showed a predominance of degenerative arthropathies. However, whereas spinal arthropathies were almost universally degenerative, a range of nondegenerative pathologies were also identified in the appendicular joints of younger animals. This difference may in part reflect the relative utility of matched clinical records. Clinical record entries both assisted interpretation of some appendicular radiographic changes and identified a range of underlying aetiologies. This was particularly the case for early developmental disease such as OC/OCD, and some traumatic appendicular arthropathies, where clinical record entries provided historical context to radiographic changes seen, clarifying both underlying aetiology and aiding interpretation of radiographic changes identified. In comparison, clinical records provided little to no assistance for interpretation of radiographic intervertebral joint disease. This was most apparent for severely affected cases involving endplate pathology. Here, due to a paucity of relevant information entered, clinical records were seldom useful in helping to discriminate between the vastly different diagnoses of discospondylitis versus degenerative disc disease. As a result, the aetiology underlying some radiographic degenerative changes seen in the spine, particularly of older lions and tigers, is still unclear.

Joint disease in the cheetah has been shown to be very different to that seen in the lion and tiger. This was evident in both the axial and appendicular skeletons. In the appendicular skeleton, the striking difference was the complete absence of appendicular joint disease in the older cheetah,

which was in stark contrast to the lion and tiger. The impact of this finding was to essentially nullify the effect that increasing age exerted on appendicular DJD prevalence for older lions and tigers. Consequently, the only significant predictor of joint disease in the appendicular skeleton was this species difference. This was very different to findings from the axial skeleton, where age alone was associated with joint disease. Here, and in contrast to the appendicular skeleton, the cheetah showed a similar age trend to the lion and tiger, with all species reporting increased spinal DJD with age. However, the radiographic distribution and appearance of axial degenerative changes differed markedly between the cheetah compared with the lion and tiger. Thus, at both skeletal levels, the cheetah was the outlier amongst the three species, however the manner in which cheetahs contrasted with the larger species was very different for the axial versus the appendicular skeleton. This suggests that for the cheetah, not only does susceptibility to joint disease differ from the lion and tiger, but different factors may contribute to the degeneration of appendicular versus intervertebral joints. For example, smaller enclosure sizes associated with urban zoos were found to be associated with both more frequent and more severe spinal DJD in cheetahs. However, this finding was not replicated for appendicular joint disease. This raises the question as to why the size of an enclosure may be associated with axial but not appendicular joint disease in this species? At the very least it appears that larger enclosure sizes do not result in misadventure and appendicular joint injuries. Beyond this, any discussion is speculation only. However, acknowledging the low detection of appendicular arthropathies in cheetah across all institutions, the failure to detect an association with enclosure size may purely be a consequence of an underpowered study, with the result reflecting an inability to determine an effect, rather than no association.

Although the prevalence of radiographic joint disease was similar between the axial and appendicular skeletons, this similarity did not apply to the severity of disease. The overwhelming finding was that more severe disease was found in the spine than the appendicular skeleton. This was particularly evident in the spines of geriatric lions and tigers, although cheetahs also could be severely affected. It is worth mentioning that the tiger was notable for severity of joint disease at all levels. These findings have important clinical implications. From the review of numerous clinical records, it was apparent that many institutions undertake annual or biannual general

health assessments for captive large cats under their care. These procedures invariably involve at a minimum the inclusion of chest and abdominal radiography. The recommendations from this study, particularly for aged lions and tigers, is that imaging of the skeleton also be included, and that providing there is no clinical indication to the contrary, that the axial skeleton be prioritised over the appendicular. However, undoubtedly, imaging both levels, or at the least inclusion of appendicular joints identified as areas of concern as per Chapter 3, is the preferred approach.

4.5 Conclusion

Whereas earlier experimental chapters of this thesis have investigated arthropathies of captive cheetahs, lions and tigers at two separate skeletal levels, in this study the skeleton has been considered as a single unit. This is both appropriate and useful, as the two levels are interdependent. The resultant larger data set not only provided an overview of joint disease in these three species, but a more holistic picture was formed, with earlier findings reinforced. Magnitudes of association were invariably increased, raising levels of confidence regarding identified correlations. As such the zoo clinician is now well equipped to focus on the identified risk factors for joint disease in these animals.

This study confirmed that joint disease is common in captive large cats, with increasing age, particularly those extended years of life seen with captive conditions, associated with an increased risk of DJD. It is now also appreciated that this age effect is a result of both increased spinal DJD for all three species, and increased age-related DJD seen in the appendicular joints of older lions and tigers. The cheetah was not only found to be singularly different to the larger species, but a higher prevalence of joint disease in cheetahs was shown to be associated with smaller enclosures. Clearly this carries significant implications when planning for species collections at urban versus open-range zoos. In contrast, tigers, particularly aged tigers, were notable for the severity of their disease at all skeletal levels.

Thus, the findings of this radiological study establish DJD as a disease of significance in captive cheetahs, lions and tigers, highlighting the need for both disease detection and monitoring of progression. However, the clinical significance of these radiographic findings is currently unknown. Certainly for other species, a disparity between the radiographic changes of DJD and the level of clinical impact is readily acknowledged. However, whether this also pertains to DJD in large cats is unclear, and is an area that requires clarification for a full appreciation of DJD in these three species.

Appendix 4.1 Frequency distribution of predictors against arthropathy status; total skeleton.

Predictor	Sub-category	Total Count	Arthropathy Negative		Arthropathy Positive	
			Number	Percent	Number	Percent
Species	Cheetah	195	153	78.5%	42	21.5%
	Lion	70	41	58.6%	29	41.4%
	Tiger	88	55	62.5%	33	37.5%
Gender	Female	152	112	73.7%	40	26.3%
	Male	201	137	68.8%	64	31.8%
Age Class	Young Adult	138	115	83.3%	23	16.7%
	Adult	100	71	71.0%	29	29.0%
	Senior	65	42	64.6%	23	35.4%
	Geriatric	50	21	42.0%	29	58.0%
Grand Total		**353**	**249**	**70.5%**	**104**	**29.5%**
Skeletal Site	Appendicular	174	132	75.9%	42	24.1%
	Axial	305	232	76.1%	73	23.9%
Grand Total		**479**	**364**	**76.0%**	**115**	**24.0%**
Cheetah Enclosure Size						
Urban vs Open Range	Open Range	134	111	82.8%	23	17.2%
	Urban	61	41	67.2%	20	32.8%
Institution A vs Other	Institution A	91	78	85.7%	13	14.3%
	Other	104	74	71.2%	30	28.8%
Grand Total		**195**	**152**	**77.9%**	**43**	**22.1%**

Appendix 4.2 Predicted (non back transformed) means and standard errors of differences, used for manual calculation of pairwise differences for significant predictors of arthropathies for the total skeleton.

Age Class	**Predicted Means**
Young Adult	-2.586
Adult	-1.633
Senior	-1.539
Geriatric	1.231
Standard errors of differences	
Average:	0.41
Maximum:	0.4713
Minimum:	0.3342

Species	**Predicted Means**
Cheetah	-1.564
Lion	-0.848
Tiger	-0.674
Standard errors of differences	
Average:	0.3948
Maximum:	0.4388
Minimum:	0.3659

Appendix 4.2 continued….

Age-Species Interaction:

Predicted Means

	Cheetah	**Lion**	**Tiger**
Age Class			
Young Adult	-3.219	-2.844	-1.754
Adult	-1.376	-1.486	-2.188
Senior	-2.061	-3.016	-0.393
Geriatric	0.326	1.949	2.95

Standard errors of differences

Average:	**0.8857**
Maximum:	1.312
Minimum:	0.5074

Appendix 4.3 P values from modelling of predictors against arthropathy status for the total skeleton. Significant associations are highlighted in red.

Predictor	**P value**
Age	<0.001
Species	0.027
Gender	0.165
Skeletal Site (axial versus appendicular skeleton)	0.292
Age Species Interaction	0.005
Species Gender Interaction	0.23
Age Gender Interaction	0.858

Cheetah Enclosure Size	Urban vs Open Range	Institution A vs Other
Enclosure Size	0.016	0.041
Enclosure Size Age Interaction	0.483	0.046
Enclosure Size Gender Interaction	0.691	0.039

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Chapter 5

A retrospective radiological study of arthropathies of captive lions, tigers, and cheetahs

Part IV: The Meniscal Ossicle and the Supinator Sesamoid Bone

5.1 Introduction

Large nondomestic felids have unique musculoskeletal anatomy, and a range of species-specific adaptations involving the appendicular joints have been recognised.[1-4] Thus, the accurate detection of arthropathies of the appendicular skeleton requires familiarisation with, and recognition of, the radiographic appearance of both these normal anatomical variants, and pathological changes affecting diseased joints.

The meniscal ossicle (also known as the sesamoid bone of the meniscus or *os meniscus* of the stifle joint) has been the focus of attention of several nondomestic felid radiological studies.[5-10] It is important for the zoo clinician to be equipped to both recognise the meniscal ossicle as such and differentiate it from other stifle intraarticular opacities such as osteochondritis dissecans (OCD) lesions, mineralisations of the caudal and cranial cruciate ligaments, and synovial osteochondromatosis.[8; 11] The radiographic presence of the meniscal ossicle has been examined in the cheetah, lion and tiger, amongst other large cat species. Collectively, some consensus has been reached, with the meniscal ossicle considered a common and normal anatomical feature of these species, developing progressively after birth as part of normal skeletal maturation. When present, it has been invariably detectable bilaterally [6; 7; 9; 10] and overwhelmingly, the conclusion is that the presence of a meniscal ossicle is not associated with degenerative changes to the stifle joint.[5-10] Speculation remains regarding a possible subspecies difference in the tiger with a failure to identify this structure in the Siberian tiger,[6; 8] and its presence in the Sumatran tiger is yet to be investigated. In addition, although age at radiographic detection of the meniscal ossicle has been determined for the tiger,[6; 8] current findings are at best an estimation for the lion,[8; 9] and age at detection remains unreported for the cheetah.

Less is known of the sesamoid bone of the elbow (referred to here as the supinator sesamoid bone), located in the tendon of origin of the supinator muscle. Although an absence of a supinator sesamoid bone has been documented for both the cheetah and lion, studies are few and the number of animals included in these studies is low.[10; 12; 13] To date, no studies have investigated this anatomical feature for the tiger. Similar to the meniscal ossicle, without knowledge that this is a normal structure, the supinator sesamoid bone could be mistaken for

pathological structures of similar radiographic description and location, such as OCD lesions of the elbow, fractured osteophytic spurs, soft tissue mineralisation and avulsion and chip fractures.[14-16]

The first goal of this retrospective study is to gain further insight into the radiographic prevalence of the meniscal ossicle and supinator sesamoid bone in tigers, lions, and cheetahs. Additionally, as the source data was derived from an investigation of degenerative arthropathies in these species, the second goal of this study was the examination of any association between the presence of either meniscal ossicles or the supinator sesamoid bone on radiography, and degenerative joint disease (DJD) of the stifle and elbow joints respectively.

5.2 Materials and Methods - A. Radiographic detection of meniscal ossicles

5.2.1 Data acquisition and scoring

Analysis for the radiographic prevalence of meniscal ossicles in captive cheetahs, lions and tigers was conducted on all stifle joint images that were assessed as eligible for inclusion for the radiological study of arthropathies of the appendicular skeleton (Chapter 3). Left and right stifle joints were considered as independent units. Each stifle radiograph was evaluated for the presence (1) or absence (0) of a meniscal ossicle, with a finding of a singular discrete focal area of meniscal mineralisation in the region of the cranial horn of the medial meniscus considered consistent with this feature.[6-10] The radiographic projection on which the mineralisation was identifiable (mediolateral or craniocaudal/caudocranial) was recorded. Identification of a meniscal ossicle in one or more projections constituted a positive finding for that stifle joint. All meniscal ossicle scoring results were then entered into a spreadsheet (Microsoft ® Excel 2016) database, with accompanying animal identification data (global accession number, species, subspecies as noted in records; tiger only, gender, date of birth, age at time of imaging; months and age class), arthropathy status of the stifle joint (0,1), and radiographic projection.

5.2.2 Data preparation for analysis

The presence of meniscal ossicles was examined according to age class at the time of imaging. As some stifle joints were imaged on multiple occasions within the same age class, repeat measures were identified and removed prior to analysis, such that each stifle joint could only appear a maximum of once per age class. The protocol used to determine the final meniscal ossicle scoring for an individual stifle joint within an age class was based on a combination of meniscal ossicle scoring and chronological order, consistent with that applied to earlier radiological studies (Chapters 2 and 3). Briefly, if no meniscal ossicle was detected, then the chronologically latest study was included. If a meniscal ossicle was detected in multiple studies within any one age class, then the chronologically earliest study was included. Where meniscal ossicle detection transitioned from negative to positive within an age class, the chronologically first study to detect a meniscal ossicle for that stifle joint was included in the data set.

The exception to one entry per stifle joint per age class was the data set constructed for an examination of agreement between mediolateral and craniocaudal projections. Here a joint that underwent orthogonal imaging within an age class, appeared twice per age class, with one meniscal ossicle score per radiographic projection. The protocol for the removal of any repeat measures was identical to that described above.

A third data set was created for the investigation of unilateral versus bilateral detection of meniscal ossicles. This data set was restricted to animals where both the left and right stifle joints were imaged within the same age class, with a meniscal ossicle detected in either or both joints. The management of any repeat measures, for both the left and right joints within an age class, was based on the protocol outlined above.

5.2.3 Statistical analysis

Descriptive statistics were used to examine the prevalence of meniscal ossicle detection in the stifle joint at an age class level. The left and right stifles were treated as independent units. Prevalence was calculated for the following factors: species, subspecies (tiger only), age class, radiographic projection, and distribution (unilateral versus bilateral). The determination of earliest age at detection utilised 'age in months' data, which was subsequently converted to 'years' (y) for reporting of results. Cross-tabulation of meniscal ossicle score against arthropathy status (negative, positive), for individual stifle joints, was performed to investigate the relationship between the detection of meniscal ossicles and arthropathy of the stifle joint. Pivot tables were used to generate tabulations that are presented as appendices.

5.2 Materials and Methods - B. Radiographic detection of the supinator sesamoid bone

5.2.4 Data acquisition and scoring

Analysis for the radiographic prevalence of the supinator sesamoid bone in captive cheetahs, lions and tigers was conducted on all elbow joint images that were assessed as eligible for inclusion for the radiological study of arthropathies of the appendicular skeleton. Therefore, data acquisition and eligibility criteria are as described in Chapter 3, with the left and right elbows considered as independent units. Each eligible elbow joint was then evaluated for the presence (1) or absence (0) of a supinator sesamoid bone, with the presence of a small, rounded or oval, discrete osseous body adjacent to, or articulating with, the craniolateral head of the radius considered consistent with this feature.[14; 15; 17] The radiographic projection (mediolateral or craniocaudal) of each elbow image, with the associated supinator sesamoid bone scoring, was recorded. Identification of a supinator sesamoid bone in one or more radiographic views constituted a positive finding for that elbow. Supinator sesamoid bone scoring results were then entered into a spreadsheet, accompanied by animal identification data as per the meniscal ossicle study, arthropathy status of the elbow (0,1), and radiographic projection.

5.2.5 Data preparation for analysis and statistical analysis

Data preparation and statistical analysis for the supinator sesamoid bone was the same as for the meniscal ossicle, with the exception that the prevalence of detection for differing radiographic projections was not calculated.

5.3 Results - A. Radiographic detection of meniscal ossicles

5.3.1 Study population

A total of 125 stifle joints from 67 animals (Figure 5.1) were analysed for the presence of a radiographically detectable meniscal ossicle. Ten stifle joints, from six animals appeared in multiple age classes. Orthogonal projections were available for 24.8% (31/125) of all stifle joints. For the remainder, 70.4% (88/125) of joints had a mediolateral, and 4.8% (6/125) of joints a craniocaudal or caudocranial projection only. Examination for unilateral versus bilateral meniscal ossicle detection was conducted on a study population of 50 animals that underwent imaging of both stifles within the same age class.

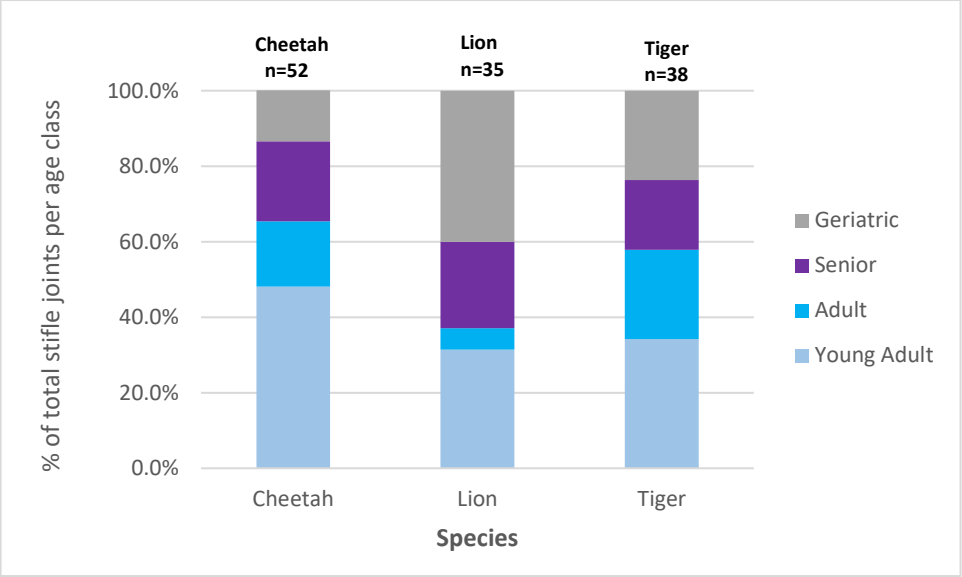


Figure 5.1 Distribution of the study population for the radiographic detection of meniscal ossicles. n= number of stifle joints in total per species

5.3.2 Descriptive analysis results

Of the 125 stifle joints assessed, 80% (100/125) showed evidence of meniscal mineralisation on radiography (Appendix 5.1). Mineralisation was most often detected in the three older age classes (prevalence 83.3 to 100%), however 65.3% of stifle joints from young adult large cats also showed evidence of meniscal mineralisation. All three species showed meniscal mineralisation in the majority of stifle joints (Figure 5.2 a-f), however prevalence was greater in the cheetah and tiger than the lion (88.5%, 81.6% and 65.7% respectively). As lions had fewer young adults compared with cheetahs and tigers, this species difference cannot be explained by age distribution. Of the 50 large cats that had both stifles imaged within the same age class, 44 had radiographically detectable meniscal ossicles in one or both stifles. Of these, 95.5% (42/44) had a bilateral distribution, and 4.5% (2/44) a unilateral distribution.

No meniscal ossicles were detected in tigers less than or equal to 1.5 y. With the exception of one aged (19.7 y) tiger, all other stifle joints from tigers of age range 3.3 to 21.8 y showed a radiographically detectable meniscal ossicle. With the exception of one cheetah (7.2 y), all cheetahs older than 1.2 y had radiographically demonstrable meniscal ossicles. Meniscal ossicles were not found in cheetahs younger than 8 months of age (0.75 y). The youngest age at detection for radiographic meniscal ossicles in the lion was 2.8 y. However, five lions within age range 2.9 to 17.8 y did not have radiographically detectable meniscal ossicles. Age ranges for meniscal ossicle detection for the cheetah, lion and tiger are shown (Appendix 5.2).

The detection of meniscal ossicles was not associated with arthropathy status. Eighty-four of 106 (79.2%) normal stifle joints showed a radiographic meniscal ossicle compared with 84.2% (16/19) of arthropathy- positive joints (Appendix 5.1). Meniscal ossicles were more frequently identified on mediolateral (98/119, 82.4%) compared with craniocaudal (20/37, 54.1%) projections. Only a minority of stifle joint studies had orthogonal views taken (31/125, 24.8%). Of these, 74.2% showed agreement in meniscal ossicle scoring between the two different views, with the most frequent finding being detection in both mediolateral and craniocaudal views. In all studies where there was disagreement in scoring, a meniscal ossicle was seen in the mediolateral but

not the craniocaudal view. Only six of 125 studies had radiographic projection restricted to a craniocaudal view. A meniscal ossicle was identified in two of six of these studies.

The prevalence of meniscal ossicles was analysed at a subspecies level for the tiger (Appendix 5.3). Fifty-five percent (21/38) of all stifle joints were from *P.tigris sumatrae*, representing 12 Sumatran tigers across four age classes. The prevalence of meniscal ossicles within the Sumatran tiger subset was 90.5% (19/21), with one of two negative stifles representing a young adult (1.3 y) tiger. Meniscal ossicles are therefore a common finding in Sumatran tigers. The lower prevalence found in white Bengal tigers (33.3%, 2/6) reflected the young age of this subset, as all four negative stifle joints were from animals less than 1.7 y (20 months) old.

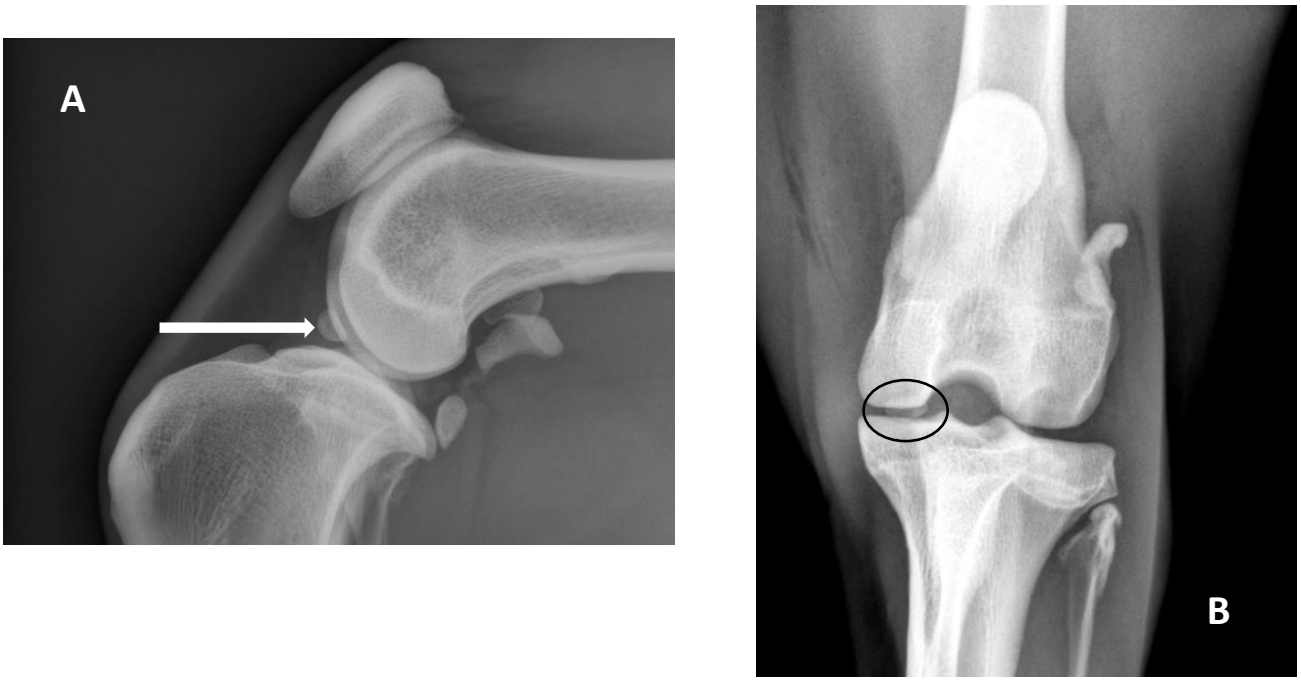
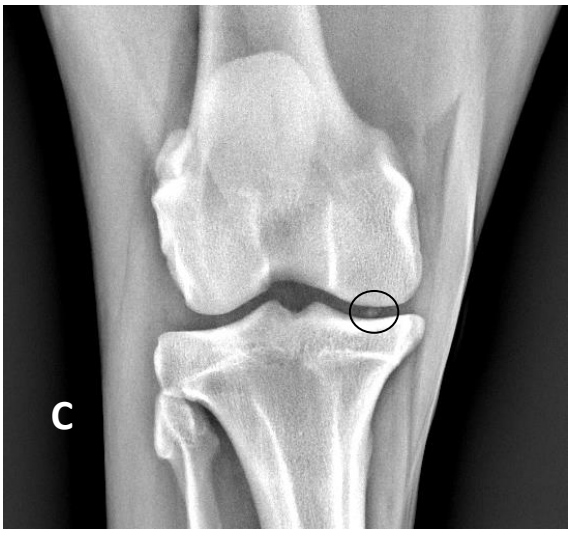


Figure 5.2a-b Mediolateral (A) and craniocaudal (B) radiographs of the stifle joint of a young adult (3.8 y) male cheetah. The meniscal ossicle is evident in both projections (arrow; lateral view, open black circle; craniocaudal view.



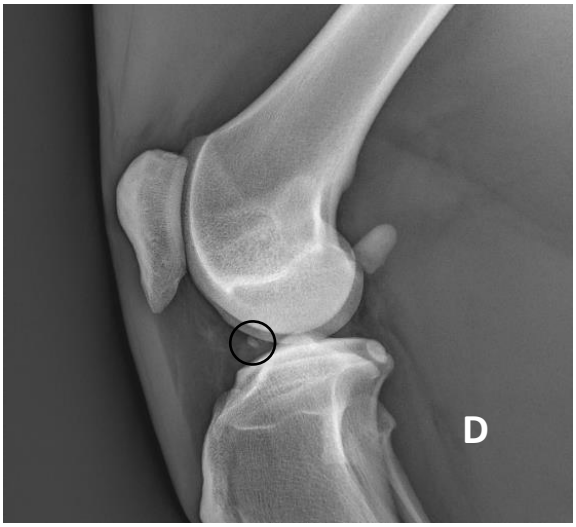


Figure 5.2c-d Craniocaudal and mediolateral radiographs of the normal stifle joint of a young adult (4.2 y) female Sumatran tiger. A small meniscal ossicle is evident in both projections (open black circle).

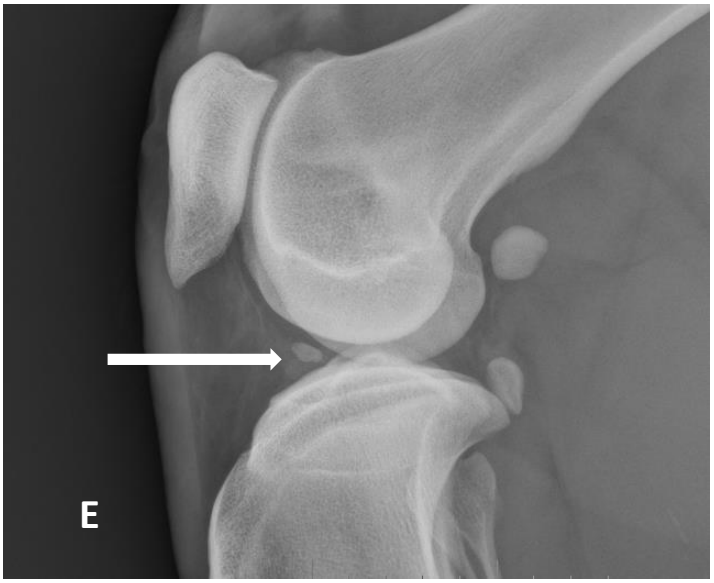


Figure 5.2e Mediolateral projection of the stifle joint of a geriatric (16.2 y) male lion. The meniscal ossicle (white arrow) is clearly demonstrated.

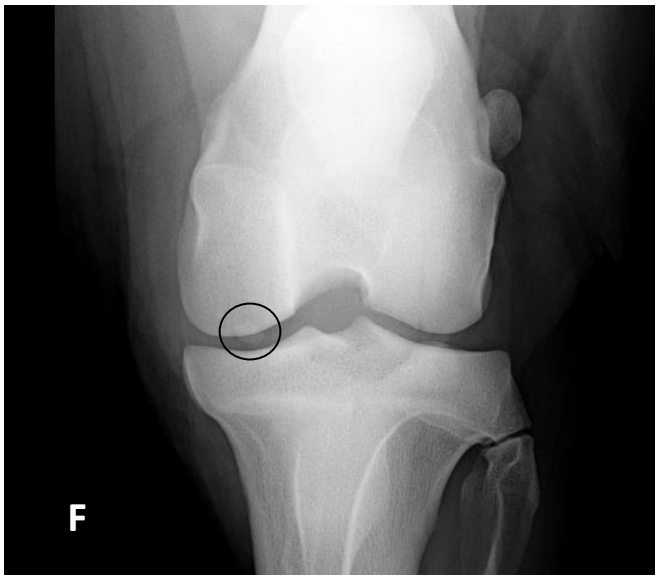


Figure 5.2f Craniocaudal radiograph of the stifle joint of a geriatric (16 y) male lion. A meniscal ossicle is evident in this view (open black circle).

5.3 Results - B. Radiographic detection of the supinator sesamoid bone

5.3.3 Study population

One hundred and two elbow joints from 53 animals were evaluated for the presence of a supinator sesamoid bone (Figure 5.3). Nine elbow joints, from six animals, appeared in multiple age classes.

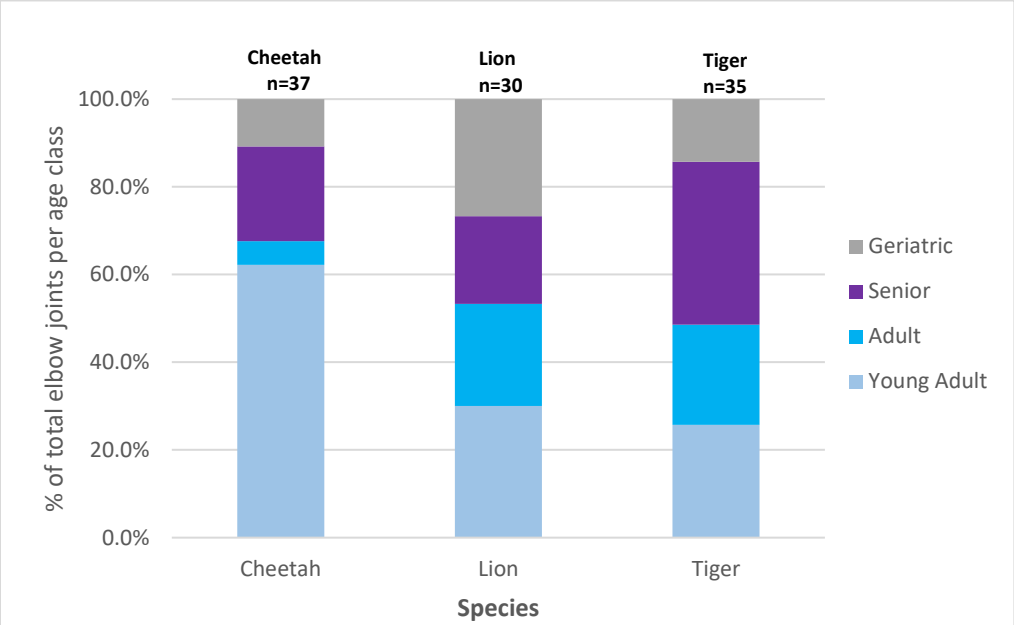


Figure 5.3 Distribution of the study population for the radiographic detection of the supinator sesamoid bone. n=number of elbow joints in total per species

5.3.4 Descriptive analysis results

Eleven of 102 (10.8%) elbow joints were found to have a supinator sesamoid bone identifiable on radiography (Appendix 5.4). Supinator sesamoid bones were exclusively associated with the elbow joints of tigers, with 31.4% (11/35) of tiger elbows, from six individual tigers, displaying this anatomical feature (Figure 5.4a-b). Senior tigers were most frequently affected (8/13, 61.5% of joints positive), however supinator sesamoids were detected in all age classes other than geriatric. The youngest age at detection for a supinator sesamoid bone in the tiger was 3.6 y, however it is noted that the study did not capture any tigers within the age range of 1.6 to 3.5 y. Of the six tigers with supinator sesamoid bones, four demonstrated a bilateral, and two a

unilateral distribution. The supinator sesamoid bone was detected on both mediolateral and craniocaudal projections, sited adjacent to the craniolateral surface of the radial head, as per domestic species.[14; 17]

The presence of a supinator sesamoid bone was not associated with arthropathy of the elbow joint (Appendix 5.5a-b). Of the 16 cases of elbow arthropathy included in the study cohort, a supinator sesamoid bone was detected in only one case (6.3%). In comparison, 11.6% (10/86) of radiographically normal elbows had an identifiable sesamoid bone in the tendon of origin of the supinator muscle. Restricting the analysis to tigers alone, 40% (10/25) of radiographically normal tiger elbows had an identifiable supinator sesamoid bone, compared with only 1% (1/10) of arthropathy-positive tiger elbows.

The prevalence of the supinator sesamoid bone was analysed at a subspecies level for the tiger (Appendix 5.6). The Sumatran tiger was overrepresented for the presence of a supinator sesamoid bone. This subspecies comprised 42.9% (15/35) of all tiger elbow joints and accounted for 81.8% (9/11) of all supinator sesamoid bones detected. In comparison, only 20% (2/10) of elbows from white Bengal tigers had a detectable supinator sesamoid bone, and no supinator sesamoid bones were detected in the elbow joints of Bengal tigers (0/4).

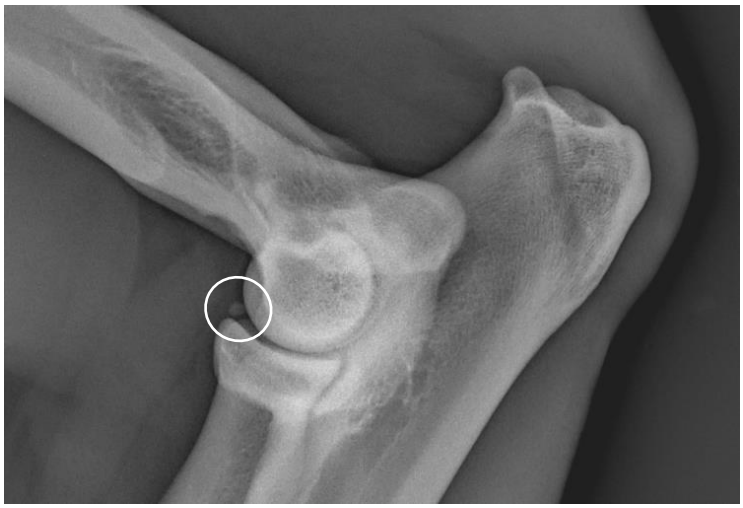


Figure 5.4a Mediolateral radiograph of the elbow joint of a young adult (4 y) female Sumatran tiger, with supinator sesamoid bone shown (open white circle) adjacent to the craniolateral radial head.

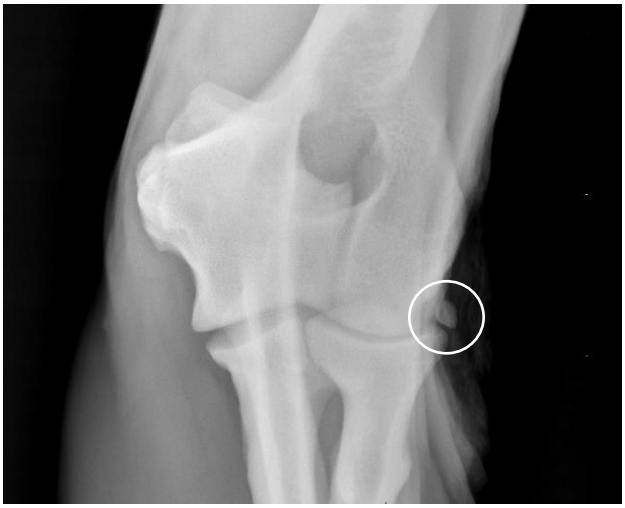


Figure 5.4b Craniocaudal radiograph of the elbow of a senior (14 y) male Sumatran tiger. The supinator sesamoid bone (open white circle) is visible on the craniolateral aspect of the head of the radius.

5.4 Discussion

The radiographic detection of meniscal ossicles has been studied for a range of large cat species,[6-10] however this is the largest such study to be undertaken for captive cheetahs, lions and tigers. This expanded data set allowed corroboration of earlier published findings, that the meniscal ossicle is a common radiographic feature in skeletally mature individuals of these three species. In fact, absence of a meniscal ossicle in the stifle joint of a mature cheetah or tiger was an unusual finding. However, such examples did exist, and in this respect this study differed from earlier and smaller studies, where one hundred percent prevalence was reported.[7; 10] In comparison, the findings from this study indicate that meniscal ossicle detection in the lion is somewhat inconsistent compared with that seen in the cheetah and tiger. Whist still a common finding, the observed prevalence reported here was lower than that for the other two species, and equally lower than that previously reported in albeit smaller studies.[8; 9] It is likely that the difference between this study's finding and previously published reports can be explained by the larger data set accessed here and that the absence of a meniscal ossicle is a normal finding in a small subset of lions.

This multi-institution data set also allowed the additional investigation of meniscal ossicle prevalence at a subspecies level for the tiger. This was a study priority, as previous research, restricted to the Bengal tiger, and singular Siberian, and Siberian Bengal hybrid animals, raised the possibility of a subspecies difference for meniscal ossicle detection.[6; 8] Our findings confirmed previous observations that meniscal ossicles can be expected in the stifles of Bengal tigers [6; 8] and show, for the first time, that meniscal ossicles are also present in Sumatran tigers.

This present study, with a large number of captive animals of known age, was in a unique position to further assess the earliest age for radiographic detection of meniscal ossicles. Not only could this study confirm previous findings that the meniscal ossicles of tigers can be detected radiographically beyond 1.7 y,[6] but for the first time the earliest age at detection for the cheetah has been reported. Whilst meniscal ossicles were detected at a younger age for cheetahs than tigers, this most likely reflects their differing developmental rates,[18] and therefore a similar stage

of skeletal maturity. However, the picture was not as well defined for lions. Although findings indicated that meniscal ossicles were not seen in animals less than 1.5 y and could be detected in lions older than 2.8 y, owing to a gap in the age range for the intervening period, and the inconsistent detection of meniscal ossicles in this species generally, further clarification was not possible and merits additional investigation.

The radiographic projection needs to be considered when evaluating the prevalence of meniscal ossicles. Although detected in both the mediolateral and craniocaudal views, the mediolateral projection was the optimal view for ease and accuracy of detection. Thus, it is this study's recommendations that any further studies investigating the prevalence of radiographic meniscal ossicles in these species, report results within the context of projections available for analysis, and that a mediolateral view be a mandatory component of future protocols for analyses of this type.

This study's findings support the theory that meniscal ossicles are not associated with radiographic features of stifle joint pathology in large cats.[6-10] However, whereas previously the meniscal ossicle has been considered a normal finding on the basis of a high prevalence in skeletally mature animals,[7; 9; 10] with verification via gross inspection of only a limited number of stifle joints,[6; 8] this study is the first to demonstrate that, for these three species, there is no relationship between the radiographic detection of meniscal ossicles and the presence or absence of radiographic DJD in the associated stifle.

Supinator sesamoid bones were not detected in this study in either the cheetah or lion. This result corroborates findings from three smaller studies that, employing a combination of radiology and dissection, failed to detect a supinator sesamoid bone in either of these species.[10; 12; 13] In contrast, this study is the first to report a supinator sesamoid bone in the tiger. It is this study's conclusion that this is a normal structure that becomes radiographically evident with skeletal maturity in this species, and that its presence is not correlated with joint disease. The increased prevalence of supinator sesamoid bones in Sumatran tigers compared with the low prevalence in white Bengal tigers and absence in Bengal tigers suggests that Sumatran tigers may have a predisposition for supinator sesamoid bone formation.

Why the tiger, and in particular certain subspecies of tiger, should have a supinator sesamoid bone, but not the cheetah nor the lion, is unknown. Previously it has been theorised that its absence in the cheetah can be attributed to an underdeveloped supinator muscle [10; 13] and decreased supination as part of an evolutionary musculoskeletal adaptation for cursorial hunting.[1] Similarly, the increased agility required for catching and grasping prey has been proposed as a reason for why the prevalence of the supinator sesamoid bone is higher for the cat than the dog.[14] However, as the lion and tiger employ similar hunting methods, with enhanced supination and pronation both reported in these species, neither theory can explain the absence in the lion. Perhaps a final theory, that a supinator sesamoid bone is beneficial for the complex anatomical movements involved in climbing,[14] may be most applicable, as tigers are more likely to climb trees than cheetahs or lions.[18-20] Imaging the elbows of leopards, which climb trees and exhibit well developed forelimb muscles of supination and pronation [1] could be used to test this hypothesis.

The sensitivity of radiography to detect a supinator sesamoid bone has been reported as dependent on the radiographic projection,[17] with a craniomedial-caudolateral oblique radiograph identified as optimal for detection in the dog. This study did not capture any elbow radiographs with this orientation. Additionally, it has been shown in the dog that even optimal positioning may not demonstrate a known supinator sesamoid bone, a failure attributed to variously the small size of the bone, disparities in joint conformation related to breed and age, and obliquity of the view.[17] This was consistent with the experience from this study, where the supinator sesamoid bone was often difficult to detect, particularly with any obliquity to the mediolateral projection. Consequently, it is likely that prevalence of a supinator sesamoid bone may be underreported here. Equally, although the supinator sesamoid bone of tigers was detectable on both standard craniocaudal and mediolateral projections, the low number of animals with this radiographic feature in this study precludes any comment regarding the relative utility of either of these projections in this species.

5.5 Conclusion

The purpose of this study was to gain further insight into two radiographically detectable, joint-associated skeletal structures, the meniscal ossicle of the stifle and the supinator sesamoid bone of the elbow, in captive cheetahs, lions and tigers. In doing so, this study has provided evidence that these structures are normal, with development occurring as part of skeletal maturation. They should therefore not be mistaken for pathological joint-associated osseous bodies.

The meniscal ossicle is a common radiological finding for skeletally mature individuals of all three species, and therefore the clinician should expect to identify this structure in the vast majority of relevant images. That they appear consistently as a small discrete singular well-circumscribed intraarticular osseous body in the region of the cranial horn of the medial meniscus, should facilitate differentiation from pathological structures in this vicinity. The ubiquitous and bilateral nature of the meniscal ossicle signifies that, if any doubt exists, imaging of the contralateral joint will assist in correct radiographic interpretation, and whilst the ossicle can be identified in both mediolateral and craniocaudal projections, the mediolateral view invariably provides a clearer appreciation of this structure.

In contrast, the supinator sesamoid bone is not found commonly. In fact, it remains unreported for the cheetah and lion, yet would be expected in approximately one third of all tiger elbows radiographed, and may have an even higher prevalence of occurrence in the Sumatran tiger. Also, in contrast to the meniscal ossicle, visualisation of the supinator sesamoid bone is highly dependent on the degree of obliquity of the radiographic projection and as a result may often remain undetected.

Appendix 5.1 Frequency of radiographic meniscal ossicle detection according to species, age class, and arthropathy status of the stifle joint.

		Meniscal Ossicle Detection		
		Stifle Total Count	**Number Stifles Positive**	**Percent Stifles Positive**
Age Class	Young Adult	49	32	65.3%
	Adult	20	17	85.0%
	Senior	26	26	100.0%
	Geriatric	30	25	83.3%
Species	Cheetah	52	46	88.5%
	Lion	35	23	65.7%
	Tiger	38	31	81.6%
Stifle Arthropathy status	Negative	106	84	79.2%
	Positive	19	16	84.2%
Grand Total		**125**	**100**	**80.0%**

Appendix 5.2 Age range (years) for the radiographic detection of meniscal ossicles in the cheetah, lion and tiger.

Species	Meniscal ossicle	Age Range
Cheetah	negative	0.6 to 7.2
	positive	1.2 to 17.6
Lion	negative	0.6 to 17.8
	positive	2.8 to 19.6
Tiger	negative	0.6 to 19.6
	positive	3.3 to 21.8

Appendix 5.3 Prevalence of meniscal ossicles in tigers at a subspecies level.

Subspecies on record	Stifle Total Count	Number Stifles Positive	Percent Stifles Positive
Bengal	2	2	100.0%
white Bengal	6	2	33.3%
hybrid	4	4	100.0%
Malayan	4	3	75.0%
Sumatran	21	19	90.5%
unknown	1	1	100.0%
Grand Total	**38**	**31**	**81.6%**

Appendix 5.4 Frequency of radiographic supinator sesamoid bone detection according to age class within species, for the cheetah, lion and tiger.

Species	Age Class	Supinator Sesamoid Bone		
		Elbow Total Count	Number Elbows Positive	Percent Elbows Positive
Cheetah	Young Adult	23	0	0.0%
	Adult	2	0	0.0%
	Senior	8	0	0.0%
	Geriatric	4	0	0.0%
Cheetah Total		**37**	**0**	**0.0%**
Lion	Young Adult	9	0	0.0%
	Adult	7	0	0.0%
	Senior	6	0	0.0%
	Geriatric	8	0	0.0%
Lion Total		**30**	**0**	**0.0%**
Tiger	Young Adult	9	2	22.2%
	Adult	8	1	12.5%
	Senior	13	8	61.5%
	Geriatric	5	0	0.0%
Tiger Total		**35**	**11**	**31.4%**
Grand Total		**102**	**11**	**10.8%**

Appendix 5.5 a-b Frequency of radiographic supinator sesamoid bone detection according to elbow arthropathy status.

Appendix 5.5a All species combined.

		Supinator Sesamoid		
		Elbow Total Count	**Number Elbows Positive**	**Percent Elbows Positive**
Arthropathy status	negative	86	10	11.6%
	positive	16	1	6.3%
Grand Total		102	11	10.8%

Appendix 5.5b Tigers only.

		Supinator Sesamoid		
		Elbow Total Count	**Number Elbows Positive**	**Percent Elbows Positive**
Arthropathy status	negative	25	10	40.0%
	positive	10	1	10.0%
Grand Total		35	11	31.4%

Appendix 5.6 Prevalence of the supinator sesamoid bone in tigers at a subspecies level.

		Supinator Sesamoid Bone		
Subspecies	**Age Class Range**	**Elbow Total Count**	**Number Elbows Positive**	**Percent Elbows Positive**
Bengal	Adult-Senior	4	0	0.0%
white Bengal	Young Adult -Senior	10	2	20.0%
hybrid	Adult	3	0	0.0%
Malayan	Geriatric	2	0	0.0%
Sumatran	Young Adult -Geriatric	15	9	60.0%
unknown	Geriatric	1	0	0.0%
Grand Total		**35**	**11**	**31.4%**

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Chapter 6

A retrospective study of the presenting clinical signs of DJD in captive lions, tigers and cheetahs

6.1 Introduction

Findings in chapters 2, 3 and 4 have demonstrated that degenerative joint disease (DJD) is commonly detected in captive cheetahs, lions and tigers, with each species showing unique radiographic characteristics of the disease. However, the clinical significance of these radiographic changes is currently unclear. This can in part be explained by the paucity of information detailing the clinical presentation of DJD in affected large cats, with published literature confined to a single case series of spinal DJD in eight captive large cats,[1] and the limited descriptions of presenting clinical signs for case studies of both axial [2-5] and appendicular [2; 6-10] DJD. There are currently no published reports of the clinical impact of axial DJD in the cheetah.

In contrast, the clinical signs of DJD in companion animals are well described. A spectrum of signs is appreciated, encompassing signs of musculoskeletal pain, loss of function and mobility impairment.[11-14] However, whilst these signs are experienced to some degree by both dogs and cats, several key differences are noted between the two species. This is most evident for the recorded signs associated with appendicular DJD. Dogs with appendicular DJD often exhibit gait abnormalities, principally as lameness, however lameness is less commonly reported for cats,[15-17] and whilst orthopaedic evaluation is a key component of canine assessment,[18] this diagnostic tool has proved unreliable for the domestic cat. Instead, alterations in activities and behaviour, including changes in walking, running, jumping and elimination habits, as well as changes in temperament, such as aggression or decreased sociability, are more commonly associated with DJD in the domestic cat. Demonstrated to either resolve completely or improve with analgesic intervention, these signs are now considered to represent the most reliable expressions of DJD-associated musculoskeletal pain in this species.[19-26]

Whilst the clinical picture for axial DJD has many similarities with that of DJD of the appendicular skeleton, potential neurological involvement creates added complexity, with associated clinical signs a reflection of the degree of disc displacement and anatomical location of the pathology. In companion animals, paresis and ataxia associated with neurological dysfunction, abnormalities on neurological examination, postural abnormalities and stiffness are all described. As with

appendicular DJD, a spectrum of activity and behavioural changes, including a reluctance to jump, abnormal elimination habits and sociability may also be reported.[14; 27-31]

Consequently, DJD in the dog and domestic cat may be associated with a broad spectrum of clinical signs. As zoo vets are often faced with a large cat that presents with one or a combination of these signs, identifying any relationship with radiographic DJD in these species will help to elucidate the spectrum of DJD-associated signs in captive large cats, and potentially streamline the diagnosis of DJD without the need for routine imaging. The aim of this retrospective study was to investigate the association between radiographic DJD status, and the recording of DJD-associated clinical signs as observed in companion animals (subsequently referred to as 'DJD-associated signs') at the time of presentation for imaging, for all cheetahs, lions and tigers that underwent skeletal radiographic imaging during the study period. Given that older large cats suffer from a range of morbidities,[32] including other neurological diseases, chronic renal disease and neoplasia, this study also investigated the association between DJD-associated signs at presentation, and the presence of non-DJD morbidities. Any age, gender or species difference in the prevalence of these clinical signs was also examined.

6. 2 Materials and Methods

6.2.1 Data acquisition and eligibility for inclusion

All radiographic studies that were eligible for inclusion for either or both the radiology scoring studies of the axial (Chapter 2) and appendicular (Chapter 3) skeleton, that had matched clinical records encompassing the imaging date, were eligible for inclusion for this study. Inclusion was independent of the recorded reason for imaging, and as a result eligible studies incorporated imaging conducted for a range of purposes, including routine health assessment. Radiographic studies that comprised only arthropathy-positive joints or axial segments, where the nature of the arthropathy was unable to be determined, were subsequently excluded from this study. As a result, 507 radiographic studies with matched records, representing 232 animals from 14 institutions were eligible for inclusion.

6.2.2 'Clinical signs of DJD' scoring system design and application

A 'clinical signs of DJD' scoring system was developed by modifying protocols previously described for the domestic cat and dog.[18; 23; 24; 26; 28; 33; 34] The DJD-associated signs represented impaired mobility, abnormal orthopaedic examination and musculoskeletal pain, reported for DJD in companion animals. The scoring system considered the following signs:

A. Signs specific to the appendicular skeleton:

I. Gait abnormalities: lameness LFL,RFL,LHL,RHL, lameness with limb not specified, stiffness, abnormal gait

II. Abnormal orthopaedic examination: muscle atrophy of the limb, joint distention/thickening, crepitus, decreased range of movement

B. Signs specific to the axial skeleton:

I. Gait and postural abnormalities: paresis, ataxia, stiffness, abnormal gait, hunched posture

II. Abnormal orthopaedic examination: paraspinal muscle atrophy, kyphosis, lordosis, scoliosis

C. Nonspecific signs of alterations in mobility:

Difficulty rising, reluctance to jump, reluctance to move, walking slowly, abnormal head carriage, reluctance or difficulty positioning to eat including bending the neck, inappropriate elimination including reluctance or difficulty positioning to urinate/defaecate.

The clinical record entries, at or associated with the date of imaging, were then reviewed for each radiographic study, with every study receiving a score (0,1) for the absence (0) or presence (1) of each of the DJD-associated signs listed above. Each radiographic study was then assigned a final clinical signs score (present (1) or absent (0)), with a positive score denoting that one or more DJD-associated signs was recorded at, or associated with, the time of imaging.

All radiographic studies with matched clinical signs scoring data were entered into a study-specific spreadsheet database (Microsoft ® Excel 2016). Patient demographic and identification data was then assigned to every radiographic study, along with date of imaging, age at time of imaging in months and age class, and the holding institution. Age class classification was as described in Chapter 2.

6.2.3 Data preparation for analysis

Each radiographic study was ascribed a radiographic DJD score of '0' (negative) or '1' (positive). A positive score was assigned if the study captured one or more DJD-positive axial segments or appendicular joints, as determined in chapters 2 and 3 respectively.

A (co)morbidity score was assigned to each radiographic study based on the dominant (co)morbidity recorded at the time of imaging. This was considered necessary due to the nonspecificity of some of DJD-associated signs, and the propensity for wild animals in general to either mask or express subtle signs only, irrespective of the nature of underlying disease. As a result, a range of morbidities experienced by captive large cats may clinically mimic DJD at presentation, and were included in this analysis.

(Co)morbidity categories included:

- no (co)morbidity (animals with no morbidities, and DJD-positive animals with no other morbidities),
- non-DJD musculoskeletal or neurological disease (including nondegenerative arthropathies, traumatic fractures, intracranial and peripheral neurological disease),
- foot or pad disease (nail disease, including overgrown and ingrown nails, worn pads, infection, trauma and pododermatitis)
- other medical or surgical conditions (all other medical or surgical morbidities, including but not restricted to renal, gastrointestinal, cardiovascular and ocular disease, and neoplasia)

Some animals underwent imaging on more than one occasion, potentially contributing multiple studies within a single age class. To avoid bias of the data, only one study per animal per age class (subsequently referred to as 'animal within an age class') was included. For those animals with multiple studies within an age class, if all studies for an animal within the same age class received a final clinical signs score of absent, the most recent study was included. If all studies received a final clinical signs score of present, the earliest study was included. Where the final clinical signs score transitioned from absent to present within age class, the chronologically earliest study to record signs was included in the data set.

6.2.4 Statistical Analysis

6.2.4.1 Inferential Statistics

Logistic regression was used to explore the association between DJD-associated signs at presentation and a range of independent predictors, for large cats that underwent a skeletal radiographic study as part of routine health care or diagnostic investigation. Univariable, followed by backward stepping multivariable logistic regression modelling was employed (Stata, version 16), leading to a 3-level random-effects logistic regression model, with 'animal within age class within institution' as the random effect. Modelling was based on a binary output for clinical signs status (absent, present) and was performed at an age class level, with the following predictors investigated: species, gender, age class, radiographic DJD status and the presence of

(co) morbidities (refer to 6.2.3). All predictors were categorical, and either nominal with several categories (species, (co)morbidity) nominal and dichotomous (gender, radiographic DJD status), or ordinal (age class).

The reference subcategory for each variable was as follows:

- cheetah (species),
- female (gender),
- young adult (age class),
- negative (radiographic DJD status)
- no (co)morbidity ((co)morbidity)

The level of statistical significance for all associations between clinical signs status and potential predictors was set at $P<0.05$. Odds ratios (OR) and P values generated from logistic regression analysis are reported, and the prob>chi2 statistic was used to assess the goodness of fit for the final logistic regression model.

In addition, a univariable 3-level random-effects logistic regression model, with 'animal within age class within institution' as the random effect, was used to examine the association between a binary output of radiographic DJD status (negative, positive) against the predictor of (co) morbidity. The level of statistical significance was as described above, and odds ratios and P values are reported. All modelling results are reported at the level of 'animal within age class'.

6.2.4.2 Descriptive statistics

Descriptive statistics were used to determine the prevalence of the final clinical signs score against all modelled predictors, and pivot tables were used to perform cross-tabulations of significant predictors from the multivariate analysis. Pivot tables were also used to report the prevalence of DJD-associated specific signs (appendicular and axial skeletons combined) versus nonspecific signs, against radiographic DJD status. All descriptive statistics were conducted according to age class.

6.3 Results

6.3.1 Study Population

Clinical records linked to radiographic studies for 324 'animals within an age class', representing 232 individual animals, were analysed for the prevalence of DJD-associated clinical signs (Figure 6.1).

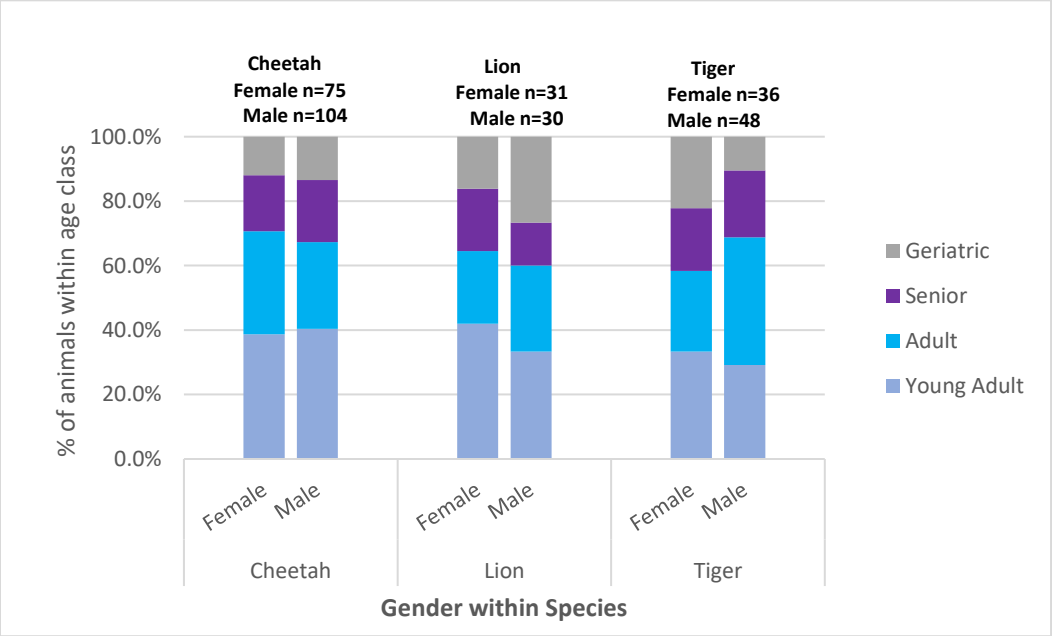

Figure 6.1 Distribution of the 'animal within age class' study population for analysis of clinical signs at presentation for large cats that underwent radiography of part or all of the appendicular or axial skeleton. n= number of individuals, gender within species

Records for 118 of the 324 (36.4%) studies were positive for one or more DJD-associated signs at the time of presentation for skeletal imaging. The frequency distribution of modelled predictors against DJD-associated signs status is shown in Appendix 6.1 and 6.2.

6.3.2 Modelling for predictors of DJD-associated signs at presentation

The most significant predictor for one or more DJD-associated signs at presentation was the presence of the (co)morbidity 'non-DJD musculoskeletal or neurological disease' (OR 70.2, P=0) followed by 'foot or pad disease' (OR 9.7, P=0.016). Radiographic DJD was also significantly associated with the recording of one or more DJD-associated signs (OR 2.6, P=0.006), as was

gender, with males twice as likely to have these clinical signs recorded compared with females (OR 2.4, P=0.007). The final model was considered a good fit (Prob > chi2 = 0.0000) and all significant results from this model are presented (Table 6.1).

Predictor	Sub-category	Odds Ratio	Standard Error	P value	95% Confidence Interval	
(Co)Morbidity	Non-DJD musculoskeletal or neurological disease	70.17547	49.41431	0	17.6526	278.9729
	Foot or pad disease	9.711321	9.124366	0.016	1.54001	61.23969
	Other medical or surgical conditions	1.570638	0.5419941	0.191	0.798627	3.088928
Radiographic DJD	Positive	2.610527	0.9053407	0.006	1.322904	5.151434
Gender	Male	2.382399	0.765171	0.007	1.269487	4.470958

Table 6.1 Table of significant results; multivariate analysis, final model.

Cross-tabulations were then performed for the three significant predictors in the multivariate analysis (Table 6.2 a-b). The effect of gender was found to be independent of both DJD status on radiography and (co)morbidity status.

In addition, as part of early model development, all independent variables under investigation were run as univariate analyses. Whilst age was found to be significant as a univariate, with geriatric animals twice as likely to show clinical signs as young adult animals (OR 2.29, P=0.03), significance was lost when run through a multivariate model. A table of univariate P values is provided (Appendix 6.3).

		(Co)morbidity							
		No (co)morbidity		non-DJD musculoskeletal or neurological disease		Foot or pad disease		Other medical or surgical condition	
Gender	Total number	Number positive	Percent positive	Number positive	Percent positive	Number positive	Percent positive	Number positive	Percent positive
Female	142	80	56.3%	18	12.7%	40	28.2%	4	2.8%
Male	182	98	53.8%	26	14.3%	52	28.6%	6	3.3%
Grand Total	324	178	54.9%	44	13.6%	92	28.4%	10	3.1%

Table 6.2a Cross-tabulations for significant predictors of DJD-associated clinical signs; gender versus (co)morbidity.

		Radiographic DJD status			
		Radiographic DJD negative		Radiographic DJD positive	
Gender	Total number	Number positive	Percent positive	Number positive	Percent positive
Female	142	110	77.5%	32	22.5%
Male	182	133	73.1%	49	26.9%
Grand Total	324	243	75.0%	81	25.0%

Table 6.2b Cross-tabulations for significant predictors of DJD-associated clinical signs; gender versus radiographic DJD.

6.3.3 Modelling the association between radiographic DJD and comorbidities

A significant association was found between radiographic DJD and the (co)morbidity 'foot or pad disease'. Univariable exploration showed that the odds of DJD detection on radiography were increased nine times (OR 8.756, P=0.004) by the presence of this morbidity. The frequency distribution of subcategories of the predictor (co)morbidity against radiographic DJD status are presented in Appendix 6.4, and the univariable table of results is presented in Appendix 6.5.

6.3.4 The prevalence of specific versus nonspecific DJD-associated signs, according to radiographic DJD status

The prevalence of DJD-associated signs specific to the appendicular and axial skeleton versus nonspecific signs of altered mobility (categories A and B versus category C, refer to 6.2.2) against radiographic DJD status, is presented in Table 6.3. For both DJD-positive and DJD-negative animals, specific signs were recorded at a higher prevalence than nonspecific alterations in mobility. Nonspecific alterations in mobility were recorded more frequently for DJD-positive compared with DJD-negative animals (25.9% versus 5.8% respectively). A higher percentage of DJD-positive animals recorded both specific and nonspecific DJD-associated signs compared with DJD-negative animals (21.3% versus 4.1% respectively).

		Specific DJD-associated signs recorded		Nonspecific signs recorded		Both specific and nonspecific DJD-associated signs recorded	
	Total Count	Number	Percent	Number	Percent	Number	Percent
Radiographic DJD negative	243	69	28.4%	14	5.8%	10	4.1%
Radiographic DJD positive	81	41	50.6%	21	25.9%	17	21.3%
Grand Total	324	110	34.0%	35	10.8%	27	8.3%

Table 6.3 Prevalence of signs specific to the axial and appendicular skeletons versus nonspecific signs of alterations in mobility, according to radiographic DJD status.

6.4 Discussion

This retrospective study is the first to investigate DJD-associated clinical signs at presentation for captive lions, tigers and cheetahs undergoing skeletal radiography. As there is little known of the clinical expression of DJD in these species, the clinical signs examined here were based on well-established criteria from companion animal medicine.[14; 20; 24; 27; 28; 35; 36] Collectively, the spectrum of signs represented mobility impairment, orthopaedic abnormalities on physical examination and musculoskeletal pain, and encompassed all clinical signs that have been reported in DJD-positive captive large cats to date.[1-10; 37] As a result, these clinical signs provided a high sensitivity, low specificity screening test to examine the association between presenting clinical signs consistent with or suggestive of DJD, and a range of predictors, including radiographic DJD and other morbidities.

This study identified a significant association between DJD-associated signs at presentation and radiographic DJD in all three large cat species investigated. Thus, the detection of any of these clinical signs should raise the index of suspicion for DJD in these species. In addition, this study's finding, that large cats presenting with both specific DJD-associated signs and nonspecific alterations in mobility were more likely to have radiographic DJD, suggests that evaluation for DJD is particularly indicated when clinical signs from both categories are reported at presentation. Significantly, despite a general acceptance that DJD is painful for a range of species,[14; 20; 38; 39] conjecture remains regarding the propensity for large cats to express musculoskeletal pain.[40] Through the inclusion of the nonspecific mobility-related items, considered indicators of musculoskeletal pain in the domestic cat,[20; 23] this study has also provided evidence that captive lions, tigers and cheetahs demonstrate pain-related behaviours, in the presence of both DJD and a range of other morbidities.

These DJD-associated clinical signs were also correlated with other morbidities, from within the categories 'non-DJD musculoskeletal or neurological disease' and 'pathologies of the foot and pad'. This is to be expected given the considerable overlap in clinical signs for all diseases that result in mobility impairment or musculoskeletal pain, and the appreciated nonspecificity of DJD-associated clinical signs in other species.[19; 41] The published literature supports this finding, with

numerous nondomestic felid papers describing DJD-like clinical presentations for conditions as diverse as vertebral malformation, traumatic patella luxation and leukoencephalopathy.[42-51] Thus, when investigating the clinical signs attributable to radiographic DJD in large cats, potential contributions from these alternative morbidity categories should be included as differential diagnoses.

Foot or pad disease was noteworthy, as not only was it associated with the clinical signs under investigation but was also associated with radiographic DJD. The most frequently reported foot/pad disease related to nail overgrowth. In contrast, pododermatitis, trauma, infection and excessive pad wear accounted for only 30% of entries. In particular, aged large cats were frequently reported to present with overgrown nails embedding into foot pads. Overgrown nails are viewed as a surrogate measure of decreased activity, and decreased activity has been shown to be associated with DJD in both domestic and large cats.[1; 20; 26] This suggests that in DJD-positive captive large cats, a feedback loop exists, whereby DJD-associated decreased activity has resulted in nail overgrowth, which in turn results in decreased activity, thus explaining the dual association between foot/pad disease, and both radiographic DJD, and DJD-associated signs. These results therefore support the recommendation that large cats presenting with overgrown nails should be evaluated for DJD.

That males were more likely to express these clinical signs than females is a finding that requires further investigation. Based on results from the radiology studies (Chapters 2-5), this is likely to be independent of radiographic DJD status, as any significant gender difference was confined to two locations in the vertebral column, of which only the lumbar spine showed higher prevalence of disease in males than females. In addition, cross-tabulations from this study identified no significant difference between males and females for the prevalence of non-DJD morbidities. The gender association may be related to a difference in morphometrics, particularly for lions and tigers, where males are considerably larger and heavier than their female counterparts.[52] Whether any sex difference persists when the focus of investigation is confined to DJD-positive animals only, remains to be determined.

This study found no association between the DJD-associated clinical signs and age or species. These results are particularly significant when considering the failure to detect radiographic appendicular DJD in older cheetahs, as reported in Chapter 3. Proposed explanations included that either DJD may not be painful in these animals, or that older cheetahs were adept at masking DJD-associated clinical signs, and thus were less likely to be presented for imaging, thereby lowering the likelihood of DJD detection. However, the findings reported here, of a clear association between DJD and the expression of clinical signs, and the recording of DJD-associated signs in cheetahs of all ages, indicate that appendicular DJD in the older cheetah is likely an unusual finding.

Finally, methodology must be considered when interpreting this study's finding that 44% of DJD-positive large cats did not present with DJD-associated signs. Whilst an incongruency between radiographic evidence and clinical impact is recognised for DJD and may have contributed,[11; 53-55] equally DJD is acknowledged as a chronic, progressive disease with often intermittent clinical expression.[1; 56; 57] As such, the restriction of clinical signs to those recorded at presentation for imaging, which was undertaken for a range of reasons, may also in part account for this finding. Whilst this aspect of study design was appropriate for this investigation, for future research investigating the clinical significance of established radiographic DJD in captive lions, tigers and cheetahs, an expanded clinical record search window that accommodates the chronic and intermittent nature of DJD-associated clinical signs is indicated.

6.5 Conclusion

This study employed a broad, highly sensitive analysis to investigate the association between the presenting clinical signs of cheetahs, lions and tigers undergoing radiography, and a range of morbidities, including radiographic DJD. Findings demonstrated that, individually or collectively, the clinical signs associated with DJD in companion animals were also found to be associated with radiographic DJD in the large cat. Representing gait and postural abnormalities, abnormal orthopaedic evaluation and nonspecific mobility impairment consistent with musculoskeletal pain, these signs show utility for further investigation of the clinical significance of DJD in these species.

Equally, these signs were not specific to DJD, but were also strongly associated with other morbidities, in particular non-DJD musculoskeletal or neurological disease. Consequently, for captive cheetahs, lions and tigers, a definitive diagnosis of DJD from presenting clinical signs alone is problematic, and the potential contributions of other morbidities to the clinical picture must also be investigated. However, consideration of the range of clinical signs reported at presentation can assist the zoo clinician, with DJD-positive large cats more likely to present with both specific gait and orthopaedic examination abnormalities and nonspecific mobility-related impairment. Notably, the detection of overgrown nails in a large cat patient should raise the clinical index of suspicion for DJD.

Appendix 6.1 Frequency distribution of predictors against DJD-associated signs status.

			Clinical signs Absent		Clinical signs Present	
Predictor	**Sub-category**	**Total Count**	Number	Percent	Number	Percent
Species	Cheetah	179	125	69.8%	54	30.2%
	Lion	61	31	50.8%	30	49.2%
	Tiger	84	50	59.5%	34	40.5%
Gender	Female	142	104	73.2%	38	26.8%
	Male	182	102	56.0%	80	44.0%
Age Class	Young Adult	120	80	66.7%	40	33.3%
	Adult	95	69	72.6%	26	27.4%
	Senior	60	35	58.3%	25	41.7%
	Geriatric	49	22	44.9%	27	55.1%
Radiographic DJD	Negative	243	170	70.0%	73	30.0%
	Positive	81	36	44.4%	45	55.6%
(Co)Morbidity	No (co)morbidity	178	136	76.4%	42	23.6%
	Non-DJD musculoskeletal or neurological disease	44	3	6.8%	41	93.2%
	Foot or pad disease	10	2	20.0%	8	80.0%
	Other medical or surgical conditions	92	65	70.7%	27	29.3%
Grand Total		**324**	**206**	**63.6%**	**118**	**36.4%**

Appendix 6.2 Frequency distribution of age class within species, against DJD-associated signs status.

Species	Age Class	Total Number	Clinical signs Absent		Clinical signs Present	
			Number	Percent	Number	Percent
Cheetah	Young Adult	71	52	73.2%	19	26.8%
	Adult	52	40	76.9%	12	23.1%
	Senior	33	18	54.5%	15	45.5%
	Geriatric	23	15	65.2%	8	34.8%
Cheetah Total		**179**	**125**	**69.8%**	**54**	**30.2%**
Lion	Young Adult	23	11	47.8%	12	52.2%
	Adult	15	9	60.0%	6	40.0%
	Senior	10	7	70.0%	3	30.0%
	Geriatric	13	4	30.8%	9	69.2%
Lion Total		**61**	**31**	**50.8%**	**30**	**49.2%**
Tiger	Young Adult	26	17	65.4%	9	34.6%
	Adult	28	20	71.4%	8	28.6%
	Senior	17	10	58.8%	7	41.2%
	Geriatric	13	3	23.1%	10	76.9%
Tiger Total		**84**	**50**	**59.5%**	**34**	**40.5%**
Grand total		**324**	**206**	**63.6%**	**118**	**36.4%**

Appendix 6.3 P values from modelling of predictors against DJD-associated signs status; univariate analysis.

Predictor	Sub-category	P value
Species	Lion	0.433
	Tiger	0.931
Gender	Male	0.007
Age Class	Adult	0.26
	Senior	0.592
	Geriatric	0.03
Radiographic DJD	Positive	0.001
(Co)Morbidity	Non DJD musculoskeletal or neurological disease	0
	Foot or pad disease	0.007
	Other medical or surgical conditions	0.382

Appendix 6.4 Frequency distribution of (co)morbidity against radiographic DJD status.

			Radiographic DJD negative		Radiographic DJD positive	
Predictor	**Sub-category**	**Total Count**	**Number**	**Percent**	**Number**	**Percent**
(Co)Morbidity	No (co)morbidity	178	146	82.0%	32	18.0%
	Non DJD musculoskeletal or neurological disease	44	32	72.7%	12	27.3%
	Foot or pad disease	10	3	30.0%	7	70.0%
	Other medical or surgical conditions	92	62	67.4%	30	32.6%
Grand Total		324	243	75.0%	81	25.0%

Appendix 6.5 Association between radiographic DJD status and the predictor (co)morbidity; univariate analysis.

Predictor	Sub-category	Odds Ratio	Standard Error	P value	95% Confidence Interval	
(Co)Morbidity	Non DJD musculoskeletal or neurological disease	.8977587	.3991726	0.808	.3755673	2.146009
	Foot or pad disease	8.755897	6.527618	0.004	2.031049	37.74686
	Other medical or surgical conditions	1.83257	.5616468	0.048	1.005042	3.341467

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Chapter 7

Summary of findings and future directions

7.1 Background

At the onset of this investigation of degenerative joint disease (DJD) in captive cheetahs, lions and tigers, whilst DJD was identified as a disease of concern in captive large cats,[1] the evidence to support this statement was sparse. Although radiography remains the most widely implemented diagnostic tool for DJD in veterinary medicine,[2-4] little was known of the prevalence and radiographic features of DJD in large cats, nor the factors that influenced radiographic DJD detection. Information regarding the clinical signs of DJD in captive large cats was similarly limited, and although incongruency between radiographic changes and clinical signs of DJD is recognised in other species,[5-10] whether this also pertained to captive large cats had not been investigated. In particular, amongst large cats, there was a paucity of information regarding DJD in the cheetah.

7.2 Research Objectives and Process

To address these knowledge gaps, this research focused on radiographic findings and recorded clinical signs for captive cheetahs, lions and tigers that underwent skeletal radiography, either for diagnostic investigation or as part of routine health care. The major objectives of this research were to provide clinically applicable results to assist zoo veterinarians in both the radiographic and clinical diagnosis of DJD for captive large cats under their care. The retrospective radiology study aimed to determine the radiographic prevalence, distribution, and characteristics of DJD for these three species of nondomestic felids, and investigate potential predictors of DJD development. Following, the investigation of DJD-associated signs as observed in companion animals, for all captive lions, tigers and cheetahs that underwent skeletal imaging, aimed to identify a suitable spectrum of presenting clinical signs for DJD in the captive large cat.

Thirteen zoos and one referral veterinary teaching hospital from Australia, New Zealand and North America contributed case material for this research, including radiographic studies and matched clinical records for all lions, tigers and cheetahs that underwent imaging during the years 1979-2019. The retrospective radiology study was conducted first, determining the prevalence and features of radiographic arthropathies, with emphasis on degenerative arthropathies, for the three species. These radiographic results were then carried forward and

matched with clinical record entries, for the retrospective study investigating the clinical signs of radiographic DJD. As a result, this is the largest investigation of radiographic DJD, and its clinical presentation, in these species to date.

7.3 Major Contributions

In Chapter 2, a retrospective radiological study of arthropathies of the axial skeleton, axial arthropathies were found to be common and almost exclusively degenerative in nature, with intervertebral joint disease and axial DJD reported for the first time in captive cheetahs. For all three species, DJD was frequently multifocal, with rising prevalence associated with increasing age. However, each species showed distinct patterns of disease. Whilst the lion and tiger both showed a propensity for severe cervical pathology in geriatric animals, the tiger was notable for the most severe and widespread axial pathology. In contrast, the cheetah was characterised by a predominantly lumbar distribution of mild to moderate pathology only. Significantly, cheetahs held at urban zoos demonstrated both a higher prevalence, and more severe axial disease, than their counterparts in open-range facilities. Of the radiographic features identified, sacroiliac osteophytosis, facet joint osteoarthritis (OA) and radiographic endplate lucency were all reported for the first time in these species.

A comparable investigation was then conducted for the six main appendicular synovial joints, presented in Chapter 3, the retrospective radiological study of arthropathies of the appendicular skeleton. Here, whilst joint disease was once again found to be common and predominantly degenerative in nature, cheetahs recorded a significantly lower level of disease than either the lion or tiger. However, this was not apparent in the younger age classes, where all species were susceptible to a range of joint insults, that in some cases transitioned to a degenerative state. In particular, the developmental diseases osteochondrosis (OC) and osteochondritis dissecans (OCD) were identified in sites previously unreported in these species. Despite these similarities as younger animals, the three species showed markedly differing patterns of disease as they aged. Whilst the lion and tiger showed a more typical, age-related increased prevalence of degenerative disease, the distribution of DJD differed between the two species, with the stifle preferentially affected in the lion, compared with the coxofemoral and elbow joints of the tiger.

Yet again, the most severe radiographic DJD was seen in tigers. In contrast, there was a conspicuous absence of any appendicular joint disease in older cheetahs. This was an unexpected finding, raising the questions as to whether cheetahs are less susceptible to appendicular DJD in advancing age, and what clinical impact if any, persisted from the early-onset DJD detected in younger animals.

In Part III of the radiology study, presented in Chapter 4, the skeleton was considered as a single unit, an appropriate approach due to the interdependency of the axial and appendicular skeletons. That thirty percent of all large cats imaged had radiographic evidence of joint disease at one or more sites reinforced earlier conclusions of the significance of joint disease in these animals. Additionally, the resultant larger data set increased the capacity to detect significant predictors of DJD development, establishing increasing age as the strongest predictor of radiographic DJD detection in the captive cheetah, lion and tiger, and confirming the lower level of radiographic joint disease in the cheetah. The earlier finding, of a potential protective effect on DJD development in cheetahs from larger enclosure sizes seen with open-range facilities, was also reinforced. The closing section of this chapter was dedicated to a comparison of joint disease in the axial versus the appendicular skeleton. Whilst there were many similarities, the most striking difference was the severity of disease, with axial DJD considerably more severe than that identified in the appendicular skeleton, a finding best demonstrated in the spines of geriatric lions and tigers, although cheetahs also could be severely affected.

As large nondomestic felids have unique musculoskeletal anatomy, it is important for zoo clinicians to be familiar with the radiographic appearance of normal anatomical variants to avoid mischaracterisation as pathological features. The final chapter of the radiological study, Chapter 5, focused on two such structures, the meniscal ossicle and the supinator sesamoid bone. Whilst both structures have been previously investigated, studies have been small,[11-17] and consequently critical knowledge gaps remained. In comparison, this research provided the largest data set to date for further evaluation of the prevalence and significance of these features. Findings from this study both confirmed that the meniscal ossicle is a normal and common radiographic feature in skeletally mature individuals of all three species,[11-14; 17] and

demonstrated for the first time the detection of meniscal ossicles in the Sumatran tiger. Unlike reports for the domestic cat,[18] no relationship was found between the radiographic detection of a meniscal ossicle and DJD of the associated stifle. Earlier reports of an absence of a supinator sesamoid bone in the cheetah and lion were also corroborated,[15-17] however this is the first study to report the presence of this structure in the tiger. Whilst not as commonly detected on radiography as the meniscal ossicle, the conclusion of this study is that the supinator sesamoid bone also is a normal structure in the tiger that becomes radiographically evident with skeletal maturation, with its presence independent of radiographic DJD status of the associated elbow.

In Chapter 6, a retrospective study of the presenting clinical signs of radiographic DJD in captive cheetahs, lions and tigers was conducted. A broad highly sensitive analysis, applied to all cheetahs, lions and tigers that underwent skeletal radiography, identified a spectrum of presenting clinical signs associated with radiographic DJD. Individually, these clinical signs represented specific findings of gait, postural and orthopaedic examination abnormalities, as well as nonspecific activity-related indicators of mobility impairment consistent with DJD-associated pain. Collectively, these are considered an appropriate set of clinical signs to apply to future investigations of DJD in captive large cats. However, these clinical signs were also associated with other morbidities, highlighting the importance of considering comorbidities when attributing clinical signs to the DJD state. Of particular relevance was the result that older cheetahs were recorded as demonstrating one or a combination of these clinical signs. This finding both addresses and refutes earlier speculation that the absence of radiographic appendicular DJD in older cheetahs may reflect a heightened ability to mask clinical signs associated with mobility impairment and musculoskeletal pain, and thereby strengthens the suggestion that appendicular DJD in older cheetah is an unusual finding.

7.4 Limitations

The main limitations of this research centre on the retrospective nature of studies, and the insensitivity of plain radiography to both detect early-stage DJD, and differentiate degenerative from nondegenerative arthropathies.[2; 6; 19] Radiographic interpretation was further complicated by the paucity of information related to both normal radiographic anatomy, and the spectrum of

degenerative changes appreciated for nondomestic felids. Additionally, as not all radiographic parameters of DJD lend themselves to objective assessment,[20] a degree of subjectivity remained when interpreting radiographic changes.

The limitations of plain radiography were compounded by the challenges posed by retrospective and opportunistic data acquisition from multiple institutions, over a prolonged time frame. Comprehensive skeletal coverage was restricted to a few cases only, and opportunistic data collection resulted in over- and underrepresentation of different skeletal sites. There was no standardisation of radiographic technique, and orthogonal projections were only infrequently available. Whilst the advent of digital imaging improved both the number and resolution of available images, the availability and quality of archived film varied substantially between institutions. The net result was a potential underreporting of DJD and a compromised investigation of disease distribution. As radiographic findings were rarely validated by alternative diagnostic tools, the nature of some radiographic degenerative changes remain unclear, and the level of insensitivity of plain radiography for DJD detection in these species unknown. Therefore, as with any retrospective opportunistic study, these results cannot claim to reflect the prevalence or distribution of DJD in the wider population of captive-held cheetahs, lions and tigers.

The study of any association between radiographic evidence for DJD and the expression of clinical signs is challenging,[21] and this research proved no exception. As with the radiology study, the retrospective and opportunistic nature of data collection impacted the investigation of clinical signs. Results were predicated on the accuracy and completeness of entries in historical records, and in this respect, this research encountered an array of limitations. Variability in the complexity and completeness of clinical records, differing terminology, and differences in both clinical conclusions and diagnostic capabilities were all problematic. In addition, the subjectivity of mobility and pain assessment, and the difficulties in performing neurological and orthopaedic evaluation in these animals, meant that the absence of standardised protocols for clinical observations, and neurological and orthopaedic assessments, was particularly limiting.

Finally, common to all studies, and a limitation frequently encountered in zoo animal medicine,[22] was the perils of small data sets. Whilst the participation of multiple institutions allowed the

creation of larger data sets, for more specific analyses, data fragmentation resulted in some studies being underpowered, with the ability to detect significant differences adversely impacted.

7.5 Findings in Summary

In summation, this research has established DJD as a significant disease in captive lions, tigers and cheetahs, and described for the first time degenerative arthropathies of the spine in captive cheetahs. With the exception of appendicular DJD in the cheetah, prevalence was shown to increase with age, chiefly in those extended years of life experienced with captive conditions. The most severe disease was found in the axial skeleton, particularly evident in the spines of geriatric lions and tigers. Whilst each species demonstrated its own unique pattern of disease, the lion and tiger showed many similarities. In contrast, the cheetah was the outlier, demonstrating significantly less disease than the larger species. This was notably evident within the appendicular skeleton, where the complete absence of appendicular DJD in older cheetahs suggests that this species has reduced susceptibility to age-related appendicular DJD. In addition, large enclosure sizes may exert a protective effect on DJD development in captive-held cheetahs, a finding with significant implications for the future management of this species in captivity. This research has also been the first to establish a spectrum of DJD-associated presenting clinical signs for these species, which also show utility for future investigations of the clinical impact of DJD in captive cheetahs, lions and tigers.

7.6 Clinical Relevance

This thesis provides critical and clinically relevant information for the zoo clinician regarding the prevalence, distribution, severity and clinical presentation of radiographic DJD in captive cheetahs, lions and tigers. In addition, the clinician is now well equipped to focus on the identified risk factors for joint disease in these animals. The high prevalence and multifocal distribution of DJD has highlighted the need to include comprehensive skeletal radiography in general health assessments for susceptible individuals. To minimise time under general anaesthesia, particularly for vulnerable geriatric animals, recommended imaging protocols to maximise detection have been provided for both the axial and appendicular skeleton.

The difficulty of DJD diagnosis from clinical signs is well accepted. This research has provided zoo clinicians with a spectrum of clinical signs to apply when both screening for DJD or determining the clinical significance of identified radiographic DJD. In addition, the findings that overgrown nails were significantly associated with radiographic DJD, and that DJD-positive animals were more likely to present with a combination of specific gait, postural and orthopaedic abnormalities, and nonspecific mobility alterations, should raise the index of suspicion for DJD, thus further assisting the clinician in DJD diagnosis.

7.7 Future Studies

Whilst logistically challenging to conduct, prospective, longitudinal radiological survey studies using standardised protocols are required to fully appreciate the scope of radiographic DJD in captive large cats. Studies should be species-specific, with the number of included animals sufficiently high to generate statistically robust results. Ongoing investigation for appendicular joint disease in the older cheetah is a particular priority. In addition, further studies are needed to clarify the association between urban enclosures and axial DJD in the cheetah, and investigate underlying factors influencing any identified impact of enclosure size on DJD in this species. Given that human studies have demonstrated links between activity levels and joint health,[23; 24] and increased opportunities for naturalistic behaviours have been proposed to exert a protective effect on the joints of captive tigers,[25] future studies focusing on cheetahs, and the relationship between enclosure size, activity levels and expressions of naturalistic behavior are needed.

Validation of imaging findings, including gross inspection and histopathological evaluation, should be incorporated into future study design. This will not only improve radiographic interpretation but allow investigation of any incongruency between radiographic appearance, gross and histological changes and clinical expression of disease. Clarification of radiographic endplate lucency is much needed. In the research presented here, discrimination between relative lucency, discospondylitis and degenerative disc disease, from either radiographic appearance or clinical presentation, was not possible. However, the differentiation is important, as unlike degenerative disc disease, discospondylitis is a treatable and somewhat reversible disease. If discospondylitis is confirmed, further research to clarify the clinical presentation is

indicated, as notably there were no entries of discospondylitis in the clinical records of these animals. This suggests that, if large cats are indeed affected with discospondylitis, they either experience subclinical disease or have an atypical presentation and clinical course.

Future research should also aim to include advanced imaging modalities, such as computed tomography (CT) and magnetic resonance imaging (MRI). Whilst not widely or easily accessible to zoo clinicians, their use in a research setting offers improved quality and faster accession of more detailed information, with notable advantages for the assessment of soft tissue pathology such as intervertebral disc disease.

Whilst this research has identified a spectrum of presenting DJD-associated clinical signs for large cats, DJD is well recognised as a chronic progressive disease with often intermittent flare ups of clinical signs.[26-28] As a result, future studies investigating the clinical significance of identified radiographic DJD in captive lions, tigers and cheetahs, if retrospective in design, should draw on clinical record entries from an expanded time frame, ideally spanning one to two years both preceding and following radiographic detection. In addition, prospective standardised studies are required to clarify the spectrum of specific clinical signs detected by direct veterinary examination, including altered gait and posture, and abnormal findings from orthopedic evaluation. Equally, further investigation of the more subjective signs, categorised in this research as nonspecific alterations in mobility, is indicated. Adaptation of one of the many validated questionnaire models applied to assess DJD-related mobility impairment and pain in companion animal medicine[7; 29-32] could be used. Employing prospective standardised methodology used by blinded assessors, these customised questionnaires allow an individualised evaluation of patient DJD-associated mobility impairment and pain and would provide capacity for input from both zoo veterinarians and keepers.

In conclusion, this thesis makes a substantial contribution to the current understanding of the scale, scope and clinical presentation of DJD in three species of captive large cats, the lion, tiger and cheetah. Yet continued investigation is required, and it is envisaged that the findings presented in this thesis will provide both impetus, and a platform, for further research in this area. Interinstitutional collaboration will be critical, and the full armory of diagnostic tools will

be required, to both optimise detection and fully appreciate the clinical impact of this multi-faceted and complex disease.

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