Getting to the heart of the matter:

An investigation into great ape mortality and cardiovascular disease

VICTORIA STRONG

Thesis submitted to

The School of Veterinary Medicine and Sciences,

The University of Nottingham

For the degree of

Doctor of Veterinary Medicine

Submitted November 2017

Abstract

Great apes housed in zoological collections have an important role to play in conservation. A sound understanding about their health and welfare forms a critical part of their custodianship. Chapter 1 of this thesis outlines a systematic review of 189 published articles relating to the topic of great ape morbidity and mortality (Strong et al. 2016). It concluded that there was a critical need for an up-to-date review of zoo-housed great ape mortality, especially among the European population, to be carried out. Such a review of data relating to 681 great ape deaths was therefore performed and is outlined in Chapter 2 of this thesis. This mortality review identified the main causes of death within each taxa and age group, and allowed for a series of recommendations about future disease investigation and monitoring to be generated. Diseases of the cardiovascular system specifically, were identified as being associated with significant proportional mortality. Despite this, however, understanding about the epidemiology, pathogenesis and diagnosis of cardiovascular disorders among great apes remains poor. The remainder of the thesis therefore outlines a series of further projects and studies designed to confront this lack of knowledge and understanding: Chapter 3 focuses on cardiovascular disease epidemiology and identifies similarities and differences in disease risk between the taxa, highlighting age and male sex as potential risk factors. Chapter 4 is dedicated to the development of two protocols designed to standardise both the ante- and post-mortem investigation of cardiovascular disease in great apes. Chapter 5 addresses the controversial topic of carrying out cardiovascular disease screening in immobilised animals by comparing the effects of two anaesthetic protocols. Finally, Chapter 6 outlines a detailed study of great ape cardiovascular pathology and specifically idiopathic myocardial fibrosis in chimpanzees. The findings of each of the studies outlined in this thesis are informative, not only for the day-to-day management of zoo-housed great apes, but also for future research into their health, disease and therefore welfare.

For my sister, Lizzy

Who taught me the importance of people and time, and without whom I would not appreciate the fullness of life nor the true value of the opportunities afforded to me through this doctorate

Acknowledgements

I would like to take this opportunity to thank the University of Nottingham School of Veterinary Medicine and Science for funding this degree. I would also like to express my gratitude to them and Twycross Zoo for affording me the chance to fulfil my childhood dream and ambition, and for providing me with many additional fantastic opportunities and unforgettable experiences over the last four years. Thanks especially to my supervisors, Kate White, Malcolm Cobb, Kerstin Baiker and Sharon Redrobe for their fantastic support and guidance, without which I would not have been able to complete the doctorate nor this thesis. A special mention also to Mike Martin for his time, expertise and excellent teaching about all things cardiology related and the Twycross Zoo veterinary and ape keeping teams; I couldn't have done it without you!

I am also grateful to the species co-ordinators and other members of EAZA Great Ape TAG committee for their input into my research and for their encouragement and belief in the project. I am also extremely grateful to all zoos, veterinarians and pathologists who have contributed samples, data or their time to my research. Special thanks go to the veterinary department at Kolmården Zoo for their involvement and participation in the anaesthesia study presented in Chapter 5. I am also extremely grateful for the help of Prof Mary Sheppard, whose input into the work outlined in Chapter 4 and 6 of this thesis has been invaluable. There are also many additional collaborators, colleagues and peers with whom I have worked closely over the past four years, and to whom I owe great thanks for their ongoing support and encouragement; there are far too many to name specifically, but they know who they are!

It's fair to say the last four and a bit years have not been easy for me, professionally or personally, and so I would like to thank my husband Ash for being there to not only celebrate the highs but to support me through the lows; I am glad to have travelled every twist and turn of this journey with him by my side. Thanks also to all my friends and family, who again have been an endless source of support and encouragement. Not least, I thank my parents for their invaluable and ongoing support but also for instilling me with the 'can do' attitude, determination and strong work ethic that this doctorate and my achievements so far have required of me.

List of publications

Published:

Strong, V. et al., 2016. A systematic review of the literature relating to captive great ape morbidity and mortality. *Journal of Zoo and Wildlife Medicine*, 47(3), pp.697–710. (Appendix 17)

Strong, V.J. et al., 2017. A retrospective review of western lowland gorilla (*Gorilla gorilla gorilla*) mortality in European zoological collections between 2004 and 2014. *Journal of Zoo and Wildlife Medicine*, 48(2), pp. 277-286 (Appendix 18)

Strong et al. 2017. A comparison of cardiovascular effects of two different anaesthetic protocols in chimpanzees (*Pan troglodytes*). Association of Veterinary Anaesthetists Spring Meeting, Manchester, United Kingdom. 26-28th April 2017 (Appendix 19)

Strong et al, n.d. A retrospective review of bonobo (*Pan Paniscus*) mortality in European and North American zoological collections between 2004 and 2014. *Currently under review with Zoo Biology*

Strong et al., n.d. Guidelines for post-mortem examination, sampling and reporting of conditions affecting the great ape cardiovascular system. *Currently under review with The International Zoo Yearbook*

Strong et al., n.d. A retrospective review of great ape cardiovascular disease epidemiology and pathology. *Currently under review with The International Zoo Yearbook*

Table of contents

List of figures

List of tables

Abbreviations

ACE	*Angiotensin converting enzyme*
Ao	*Aortic root diameter*
ARVC/D	*Arrhythmogenic right ventricular cardiomyopathy/dysplasia*
AZA	*Association of Zoos and Aquaria*
BNP	*Brain natriuretic peptide*
bpm	*Beats per minute*
BSE	*British Society of Echocardiography*
CI	*Confidence interval*
CITES	*Convention on International Trade of Endangered Species*
COD	*Cause of death*
CT	*Computed tomography*
cm	*Centimetre*
d	*Days*
DAP	*Diastolic arterial blood pressure*
DCM	*Dilated cardiomyopathy*
DOB	*Date of birth*
EAZA	*European Association of Zoos and Aquaria*
ECG	*Electrocardiogram*
EF	*Ejection fraction*
EEP	*European Endangered species Programme*
F	*Female*
f_R	*Respiratory rate*
FCM	*Fibrosing cardiomyopathy*
Fig	*Figure*
g	*Grams*
GAHP	*(The) Great Ape Heart Project*
HR	*Heart rate*
hrs	*Hours*
HCM	*Hypertrophic cardiomyopathy*
ICD	*International Classification of Diseases system*
IMF	*Idiopathic myocardial fibrosis*
IUCN	*International Union for Conservation of Nature*
IVS	*Interventricular septum/septal*
IVSd	*Interventricular septal wall thickness in diastole*
kg	*Kilograms*
LA	*Left atrial diameter*
LV	*Left ventricle*
LVIDd/s	*Left ventricular internal dimension in diastole/systole*
LVOT	*Left ventricular outflow tract diameter*

LVPW	*Left ventricular posterior wall thickness*
LDAC	*Left dominant arrhythmogenic cardiomyopathy*
M	*Male*
m/s	*Metres per second*
MAP	*Mean arterial blood pressure*
ml	*Millilitre*
mm	*Millimetre*
mmHg	*Milligrams of mercury*
MRI	*Magnetic resonance imaging*
Max.	*Maximum*
Min.	*Minimum*
Misc.	*Miscellaneous*
MMVD	*Myxomatous mitral valve degeneration*
MV	*Mitral valve*
n	*Number*
ND	*No data*
ns	*Not (statistically) significant*
NSF	*No significant findings*
PR	*Pulse rate*
PRISMA	*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*
RCM	*Restrictive cardiomyopathy*
RR	*Relative risk*
RV	*Right ventricle*
SAP	*Systolic arterial blood pressure*
SD	*Standard deviation*
SPARKS	*Single Population Analysis and Record Keeping Software*
SpO_2	*Arterial oxygen saturation*
SSP	*Species Survival Plan*
Strep.	*Streptococcus*
T	*Total*
TAG	*Taxon Advisory Group*
TV	*Tricuspid valve*
TZ	*Tiletamine-zolazepam*
TZM	*Tiletamine-zolazepam and medetomidine*
U	*Unknown/undetermined*
UK	*United Kingdom*
USA	*United States of America*
Vel.	*Velocity e.g. E' (E wave) vel., A'(A wave) vel.*
$Vel_{AV/PV}$	Peak flow velocity at the aortic/pulmonic valve
WAZA	*World Association of Zoos and Aquaria*
y/yr(s)	*Year(s)*
WHO	*World Health Organisation*

Ethical review statement:

Unless otherwise stated, the work carried out in fulfilment of this thesis was approved by the University of Nottingham's ethical review committee (Ref: 910 130711, July 2013) prior to commencement. Study protocols and any relevant documentation were also approved by the EAZA Great Ape TAG and research committee prior to use or dissemination.

INTRODUCTION

I The great apes

The term great ape *(Hominidae)* refers to a taxonomic family of primates in four distinct genera (Groves 2005):

- *Pongo*: Bornean orangutan *(Pongo pygmaeus)*; Sumatran orangutan *(Pongo abelii)*
- *Gorilla:* Eastern gorilla *(Gorilla beringei);* Western gorilla *(Gorilla gorilla)*
- *Pan:* Chimpanzee *(Pan troglodytes)*; Bonobo *(Pan paniscus)*
- *Homo:* Human *(Homo sapiens)* and near-human ancestors and relatives such as Neanderthal

Due to growing threats posed mostly by habitat loss and hunting but also the illegal pet trade, climate change and disease, wild populations of non-*homo* great apes are in rapid decline. All species are listed on the IUCN Red List of Threatened Species as either endangered (bonobos and chimpanzees) or critically endangered (Eastern and Western gorillas; Bornean and Sumatran orangutans) (IUCN 2016).

II Zoo-housed great apes in conservation

As the wild population of any species declines, the role of animals managed in zoos increases in importance. Animals housed in zoos serve not only as ambassadors for their species in helping to raise funds and public awareness of conservation issues but as a potential source for repopulation, thereby providing insurance against extinction. The maintenance of such

populations requires careful cooperative management on an international, regional and in some cases, even global scale.

More than 1,000 species and sub-species are currently managed under *ex situ* breeding programmes, the aims of which are to maintain self-sustaining, demographically stable and genetically viable populations (WAZA 2016a&b). For great apes, two main groups of breeding programmes exist: The European Endangered species breeding Programmes (EEPs), run under the auspices of the European Association of Zoos and Aquaria (EAZA) and the Species Survival Plans (SSPs), run under the auspices of the American Association of Zoos and Aquaria (AZA). Each SSP/EEP has an assigned species coordinator and species committee. The individual breeding programmes for each of the great ape species are brought together under the administration of the EAZA Great Ape and the AZA Ape Taxon Advisory Groups (TAGs). It is the role of the experts in each of these committees to maintain studbooks, carry out demographic and genetic analysis and to plan for the future management and conservation of the species (AZA 2014; EAZA 2013; WAZA 2016a).

The ultimate success of these breeding programmes depends not only on the occurrence of births, but also the rearing of animals to sexual maturity and the maintenance of a population of healthy adults of sound reproductive status (Munson & Montali 1990). Any disease threat to zoo-housed great apes is therefore of great concern not only for the individual or zoological collection affected but more widely, for the future breeding and conservation of these endangered animals.

III Zoo-housed great ape health, disease and mortality

The type and intensity of veterinary care afforded to great apes held in zoos varies greatly depending on factors such as: collection size, need or demand, funding availability, attitudes

of management and local authority stipulations. Many zoos will utilise the services of a local veterinarian, the level of specialist experience or training of whom might be quite limited. Often in these instances a 'reactive' medicine approach is taken, whereby problems are responded to as and when they occur. By contrast other, often larger zoological collections employ their own on-site vet or veterinary team. Usually these individuals will have a great deal more specialist experience and/or training and qualifications in the field of zoological medicine. Often this set up permits veterinarians to take more of a preventative approach; proactively screening for, controlling and managing illness, thereby maximising animal health and welfare.

Whatever the approach to health management employed within a zoo, veterinary, keeping and curatorial staff require a sound understanding of the diseases and disorders to which the animals they take care of are susceptible. A whole host of diseases affecting each of the body systems have been reported in the literature to affect great apes, a detailed overview of which is outside the remit of this introduction. Historically, infectious diseases especially of the gastrointestinal and respiratory tracts were the most common causes of morbidity and mortality among captive great apes (Benirschke & Adams 1980; Cousins 1983; Schmidt 1975). Many great apes, initially brought into captivity from the wild, survived for only very short periods of time. Thankfully, however, dramatic advances in animal husbandry and veterinary care have occurred and many great apes housed in zoos now live long into old age. As management practices and life expectancy continue change over time, so too do patterns of disease and mortality. In 1990, a published overview of diseases that had been reported to affect orangutans, gorillas and chimpanzees during the preceding decade (Janssen & Bush 1990) suggested this to be the case. The authors concluded that reports about age-related conditions such as gonadal neoplasia and cardiovascular disease dominated the literature at that time. There was therefore the suggestion that a shift in the diseases to which these animals most

frequently succumb had occurred. However, there are two potential criticisms of this review: Firstly, except for their statement about cardiovascular and neoplastic disorders, the authors do not quantify the relative importance of each of the conditions they described. Also, given that the paper was a narrative review with no methodology provided, it is not possible to ascertain the completeness of the information presented. There have been no reviews of the veterinary literature of great apes published since this article by Janssen and Bush. With a view to expanding upon their work, therefore, a systematic review of the zoological and veterinary literature relating to great ape morbidity and mortality between 1990 to 2014 was carried out (Strong et al. 2016). The methodology and main findings of this review are outlined in Chapter 1 of this thesis.

Whilst literature reviews are informative, it must be remembered that amount of literature coverage a disorder receives might be influenced by several factors, for example: the veterinary knowledge and diagnostic capabilities available at the time; the level of allocated funding; the specific clinical and academic interests of the author(s); or simply the author's perceived importance of the condition. Perhaps almost ironically, an author's perception of a condition's importance can in turn be influenced by the amount of literature coverage it receives. The veterinary, and especially zoological, literature comprises many individual case reports which, whilst an invaluable resource for the everyday practicing clinician, give very little perspective on the significance of a condition at a population level. An appreciation of this latter point requires in depth epidemiological study and the calculation of comparative incidence of various diseases and disorders. True prevalence figures can often be very difficult to ascertain especially in populations such as these. Proportional mortality, however, can serve as a surrogate marker and provide information about the relative significance or importance of a disease. Several reviews of mortality among great apes do exist within the literature, however they are not without limitation and are particularly lacking for the European zoo-housed great

ape population (this literature is reviewed in Chapter 1 of this thesis). For this reason, a retrospective review of mortality among the managed European zoo-housed chimpanzee, gorilla and orangutan, and combined European and North American zoo-housed bonobo populations was conducted. The methodology and main findings of this review are provided in Chapter 2 of this thesis.

IV Great ape cardiovascular disease

The topic of great ape cardiovascular disease has received significant coverage in the veterinary and zoological literature over recent years (Chapter 1). It is frequently cited as being one of the most significant causes of morbidity and mortality to affect great apes in captivity (Gamble et al. 2004; Lowenstine et al. 2008; Meehan & Lowenstine 1994; Nunamaker et al. 2012).

The term cardiovascular disease, however, does not refer to just one, but a group of non-communicable disorders affecting the heart and/or vasculature (World Health Organisation 2011). The term therefore encompasses a wide spectrum of diseases and conditions including: coronary heart disease; cerebrovascular disease; hypertension; peripheral vascular disease, rheumatic heart disease; congenital heart disease; cardiomyopathies and; heart failure. Many of these cardiovascular disorders which have long been known to affect humans and many animal species, have also been reported across the great ape taxa (Chapter 1). Despite the reported significance of great ape cardiovascular disease, however, understanding about the specific conditions as well as their aetiology and pathogenesis remains unclear.

Several limitations to progression of understanding within this field were found to exist, including:

1) A lack of standardisation with regards data and sample collection across the taxa;

2) The absence of a comprehensive searchable clinical and pathological database and
3) A lack of dedicated research in the areas of great ape heart disease aetiology, diagnosis and treatment

In 2010, the critical need for these issues to be tackled within the North American zoo community was addressed by the formal establishment of the Great Ape Heart Project (GAHP). Based at Zoo Atlanta, the GAHP is the name given to a group of dedicated and coordinated subject matter experts who, together, provide a network of clinical, pathological and research strategies to aid in furthering understanding and reducing mortality associated with great ape heart disease, mostly across North America. However, no equivalent initiative existed within Europe: zoo-based investigative efforts were fragmented and uncoordinated; there was a paucity of data and samples available for research; zoos were lacking in support and guidance and in clarity for who to turn to when in need of it, and; there was no team of personnel dedicated to furthering knowledge understanding in this field.

In 2013, a pan-European taskforce was therefore formed, bringing together experts in the fields of zoological and cardiovascular medicine, anaesthesia, pathology and molecular biology. Run under the auspices of the EAZA Great Ape TAG and directed by one of their veterinary advisors, the Ape Heart Project (based at Twycross Zoo; www.twycrosszoo.org/ape-heart-project.aspx) aims to coordinate and drive the study of cardiovascular disease across European zoological collections. The work outlined in this thesis summarises the main efforts and outputs of this initiative to date, and encompasses three main broad topic areas, namely: great ape cardiovascular disease epidemiology, clinical diagnosis and pathology.

IV.I Great ape cardiovascular disease: epidemiology

Epidemiology is *"the study of the distribution and determinants of health-related states or events (including disease), and the application of this study to the control of diseases and other health problems"* (World Health Organisation 2014). An understanding of the epidemiology of any given disease is essential to its prevention and control.

In human medicine, the main causes of cardiovascular diseases have been very well studied and include many modifiable factors such as: tobacco use, diet, alcohol consumption, physical inactivity and obesity. Non-modifiable factors which are known to influence cardiovascular disease risk are: age, gender, ethnic origin and, family history and genetic factors (Anderson et al. 1991; van den Broeck et al. 2005; D'Agostino et al. 2013; Institute of Medicine 2010; Jousilahti et al. 1999; World Health Federation 2017). In addition, being diagnosed with other health conditions such as diabetes can also increase your risk of heart disease (Fox 2010; Kannel & McGee 1979).

The risk factors for cardiovascular disease in animal species are comparatively less well studied. In companion animal species, genetics play a very important role as selective breeding for certain aesthetic or behavioural traits over the years, has led to certain breeds and family lines being particularly predisposed to certain cardiac diseases. Examples include: dilated cardiomyopathy (DCM) in the Doberman Pinscher, English Cocker spaniel and Boxer dog (Simpson et al. 2015; Tidholm et al. 2001); arrhythmogenic right ventricular cardiomyopathy (ARVC) in the Boxer dog (Basso et al. 2004; Meurs et al. 2014); myxomatous mitral valve degeneration (MMVD) in the Cavalier King Charles Spaniel (Häggström et al. 1992) and; hypertrophic cardiomyopathy (HCM) in Maine Coon cats (Kittleson et al. 1999). Given that zoo-housed great ape breeding is tightly managed as part of species conservation programmes,

the potential role of genetics and selective breeding in cardiovascular disease risk is an important topic for consideration.

Unlike humans, zoo-housed great apes do not have a diet heavy in saturated fats, nor are they exposed to the harmful effects of alcohol or tobacco smoke. Their susceptibility to heart disease despite this, is therefore of great interest. To ascertain the role that other known, and perhaps even novel, risk factors might play in the aetiopathogenesis of cardiovascular in great apes, requires more in depth epidemiological study of affected individuals. A study looking at the distribution (frequency and pattern) of cardiovascular disease occurrence in zoo-housed great apes was carried out and is outlined in Chapter 3 of this thesis.

IV.II Great ape cardiovascular disease: ante-mortem (clinical) diagnosis

In human and veterinary medicine alike, a diagnosis of cardiovascular disease is made following interpretation of information gathered from: clinical history taking (symptoms, family history); physical examination; radiography; electrocardiography (ECG); echocardiography and/or; serum cardiac biomarker assessment. More advanced diagnostic modalities such as magnetic resonance imaging (MRI), computed tomography (CT) and angiography might also be used (Kittleson & Kienle 1998; Mann et al. 2014). The ways in which the use of these tests can be extrapolated from human and domestic veterinary to great ape medicine are, however, not yet fully understood. There is also a lack of understanding about what is normal for these species and there are inconsistencies with regards the investigative approach undertaken between different zoological collections. The latter of these factors limits the quality of images and information gathered and therefore their potential use, not only in clinical decision making, but also in research. A guideline protocol for the echocardiographic assessment of the great ape heart was therefore generated with the aim of

standardising and improving the quality of image and data collection between zoological collections. The stages involved in this protocol development are outlined in Chapter 4 of this thesis.

Several great apes housed in North American zoos have been trained for conscious echocardiographic and/or blood pressure assessment (GAHP 2016). However, this is not yet commonplace among European zoological collections. When considering echocardiography specifically, the views that can be obtained even in a well-trained animal are often of limited diagnostic quality (GAHP 2016b). The attainment of a more comprehensive and detailed echocardiographic study therefore requires that the animals are anaesthetised. A variety of injectable and inhalational agents are used in the immobilisation, sedation and anaesthesia of great apes (Cerveny & Sleeman 2014). All such anaesthetic agents are likely to have some effect on the cardiovascular system and potentially therefore influence diagnostic information gathered. For this reason, a study comparing the effects of two different anaesthetic protocols on the heart in a group of healthy chimpanzees was carried out. The methodology, results and implications of this study are outlined in Chapter 5 of this thesis.

IV.III Great ape cardiovascular disease: pathogenesis

Pathology can be defined as the science of the causes and effects of diseases, and specifically the branch of medicine that deals with the laboratory examination of samples of body tissue for diagnostic or forensic purposes (Oxford University Press 2017). Specifically, macroscopic (gross) pathology is concerned with the changes associated with disease that are visible to the naked eye. Through histopathology, the disease process can also be examined at a cellular level (Kumar et al. 2013). Together, the information gleaned from pathological examination of tissues and other biological samples (blood, urine, cell cultures) can provide not only definitive

diagnoses but also information relating to the causes and pathological processes involved in disease development and progression.

However, the aforementioned issues associated with inconsistencies with regards clinical diagnosis between institutions is also true of the approach to post-mortem examination of the great ape heart and indeed also the subsequent reporting of findings (see Chapters 2 and 3). This again limits progression of understanding about the aetiopathognesis of great ape cardiovascular disease. In Chapter 4, the development of a guideline protocol for the standardised post-mortem examination and sampling of the great ape heart is outlined. Use of this protocol enabled the collection of >30 great ape hearts from zoological collections across Europe and one sanctuary in Africa. Chapter 6 of this thesis describes a pathology study in which these hearts underwent detailed macroscopic and histopathological examination using a standardised approach akin to that used in the investigation of sudden cardiac death in people.

V Aims and objectives for the thesis

Reducing mortality associated with great ape cardiovascular disease, demands a sound understanding of how it can be treated and ultimately prevented. This is, however, not possible without first improving knowledge about its epidemiology, aetiopathogenesis and diagnosis.

This thesis explores various, different but related, topics relating to great ape mortality and cardiovascular disease. The ways in which these areas of research relate to one another and to the individual chapters of this thesis, are summarised in Figure I (overleaf).

Broadly, the overarching objectives of this thesis were to:

1. ***Identify the main gaps in the literature*** *(Chapter 1)* ***and the main limitations to current understanding*** about great ape mortality *(Chapters 1 & 2)*

2. ***Identify the main causes of mortality*** among the European zoo-housed great ape populations and specifically, to ***quantify the relative importance of cardiovascular disease*** as a cause of death among each of the taxa *(Chapter 2)*

3. ***Explore the epidemiology of great ape cardiovascular disease*** and identify factors of interest for further research into its aetiopathogenesis *(Chapter 3)*

4. ***Encourage and promote a pro-active approach*** to cardiovascular disease screening and research into this topic across European zoological collections *(Chapter 4)*

5. ***Standardise the approach to ante-mortem and post-mortem examination*** of the great ape heart across European zoological collections to:

    a. Improve the quality of great ape cardiovascular disease investigation and

    b. Generate a wealth of good quality, consistent and comparable data and samples for future research in this area *(Chapter 4)*

6. ***Compare the effects of two different anaesthetic agents on the cardiovascular system*** and to evaluate their suitability to immobilise great apes use undergoing echocardiography for the purpose of cardiovascular disease screening *(Chapter 5)*

7. Utilise a standardised and consistent methodology to ***identify, describe and characterise the main conditions*** affecting the cardiovascular system of great apes and to compare these with those described in humans and domestic animal species *(Chapter 6)*

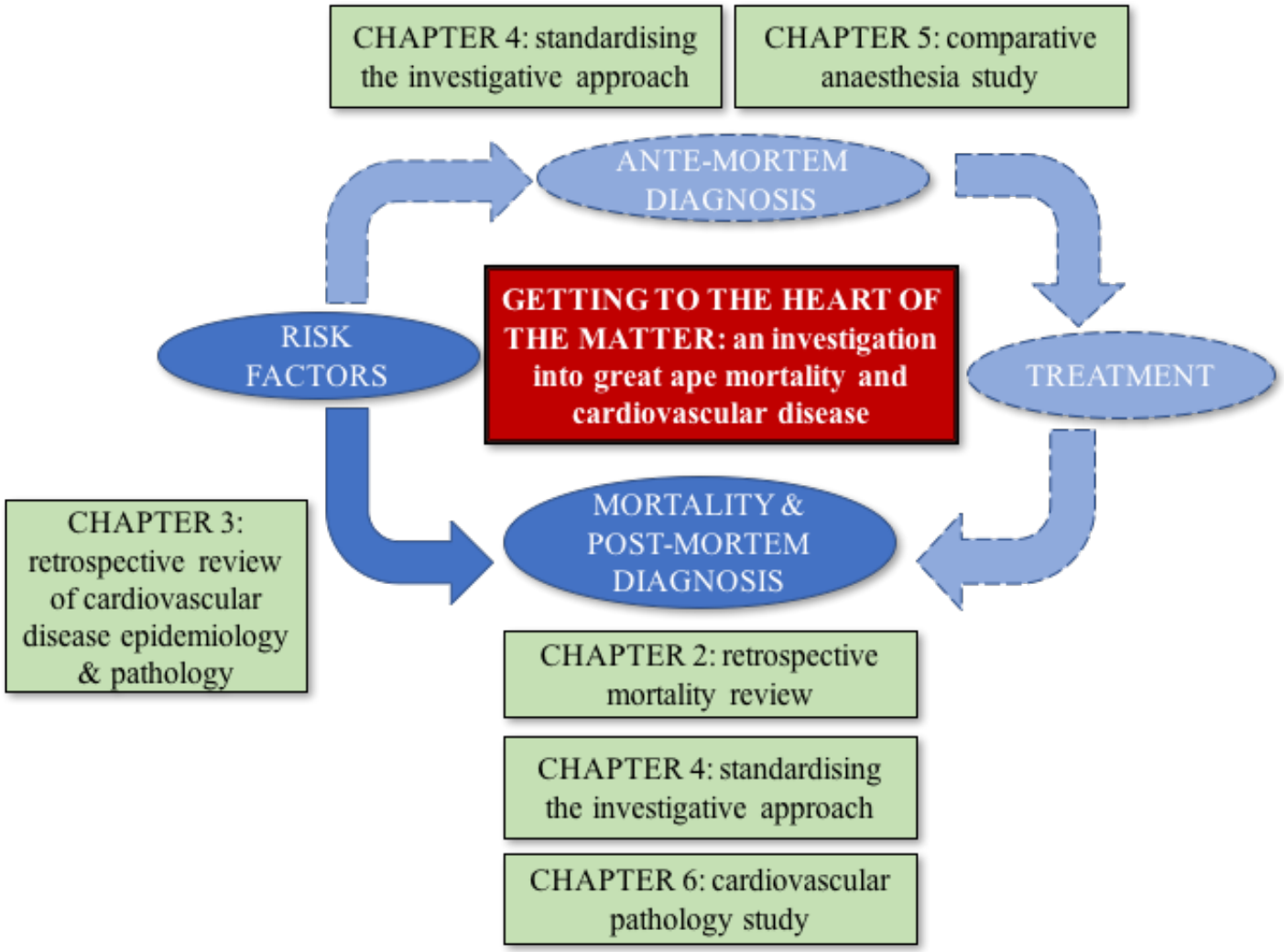


Figure I: Diagram showing the key aspects of great ape cardiovascular disease which are explored as part of this thesis, as well as the relevant chapters within which each topic is covered

CHAPTER 1:

A SYSTEMATIC REVIEW OF THE LITERATURE RELATING TO GREAT APE MORBIDITY & MORTALITY

1.1 Zoo-housed great ape health: an introduction

Zoo-housed great apes have an important role to play in *ex situ* conservation through breeding programmes, the success of which relies not only on regular births, but also the rearing of animals to sexual maturity and the maintenance of a population of healthy adults of sound reproductive status (Munson & Montali 1990). Any disease threat to zoo-housed great apes, is therefore of great concern not only for the individual or zoological collection affected but more widely, for the future breeding and conservation of these endangered animals. One of the aims of the work outlined in this chapter was therefore to review and summarise the occurrence of these such diseases, as they are reported in the veterinary and zoological literature.

The purpose of the literature review hereby presented was to identify gaps in current knowledge, and highlight the main limitations to current understanding about great ape disease and mortality. Specifically, the objectives of this systematic literature review were as follows:

1. To quantify the amount of literature coverage afforded to each of the various causes of great ape morbidity or mortality in the zoological and veterinary literature between 1990-2014 by taxa, aetiology and body system affected
2. To identify and critique papers which report upon the comparative prevalence of various diseases and disorders (morbidity and mortality reviews) within the captive great ape population

1.2 Methodology

The methodology for this systematic literature review followed published guidelines (Centre for Reviews and Dissemination 2009; PRISMA Group et al. 2009).

Eligibility criteria: The research question was defined as: "What are the main causes of morbidity and mortality among great apes kept in captivity?" The inclusion criteria were set to include papers which reported upon specific, naturally occurring, diseases or disorders of clinical relevance among captive bonobos, chimpanzees, gorillas and orangutans. The term captive was used to include those animals housed in zoological, research and sanctuary facilities in which they had no contact with wild animals. Semi-wild and pet animals were excluded.

Information sources: Databases were selected based upon their reputation, relevance and reported coverage of the literature: CAB Abstracts is reported to be the database which provides the widest coverage of veterinary journals and was selected for this reason (Grindlay et al. 2012). Also selected for its breadth of coverage was the Web of Knowledge interface which encompassed databases from a wide range of topic areas (Web of Science Core Collection, BIOSIS Citation Index, BIOSIS Previews, Current Contents Connect, Data Citation Index, Derwent Innovations Index, MEDLINE, and SciELO Citation Index). Zoological Record was searched due to its coverage of zoology specific journals not indexed elsewhere.

Search terms: Alternative search terms and synonyms for each of the key words within the research question were used (see Appendix 1). The search was limited to include only those papers that were written in English and had been published since 1990. The papers obtained from all three databases were merged and duplicates removed.

Article selection and sorting: Papers were screened for relevance (see Appendix 2) and the final list of relevant publications was exported to an electronic spreadsheet (Microsoft Excel, 2010). The content of the articles was reviewed and each classified as either: a) morbidity/mortality studies or b) case reports/series and papers reporting upon the prevalence of single diseases.

Data collection and processing: The morbidity/mortality studies were reviewed and the following data extracted from each: author, date of publication, period of study, type of study (retrospective/prospective; interventional/observational; multi/single-centre; longitudinal or cross-sectional), details relating to the study population (taxa, number of subjects, age, sex) and the main causes of morbidity/mortality identified. The reviews were assessed for risk of bias and the main limitations of each identified. The contents of the case reports/case series and single disease frequency studies were reviewed and each article categorised and coded according to: a) the taxa under study; b) the aetiology of the primary condition being reported and; c) the body system affected. Aetiological categories were adapted from the DAMNITV classification system as used by Rizzo et al. (2007) and on categories used by Mesle (1999). Body system categories were adapted from those used by Robinson et al. (2015). For complete category definitions and relevant coding see Appendices 3 & 4. Descriptive statistics were used to analyse the data and the amount of literature coverage afforded to each of the categories was calculated. The main diseases and disorders reported within the literature were also identified.

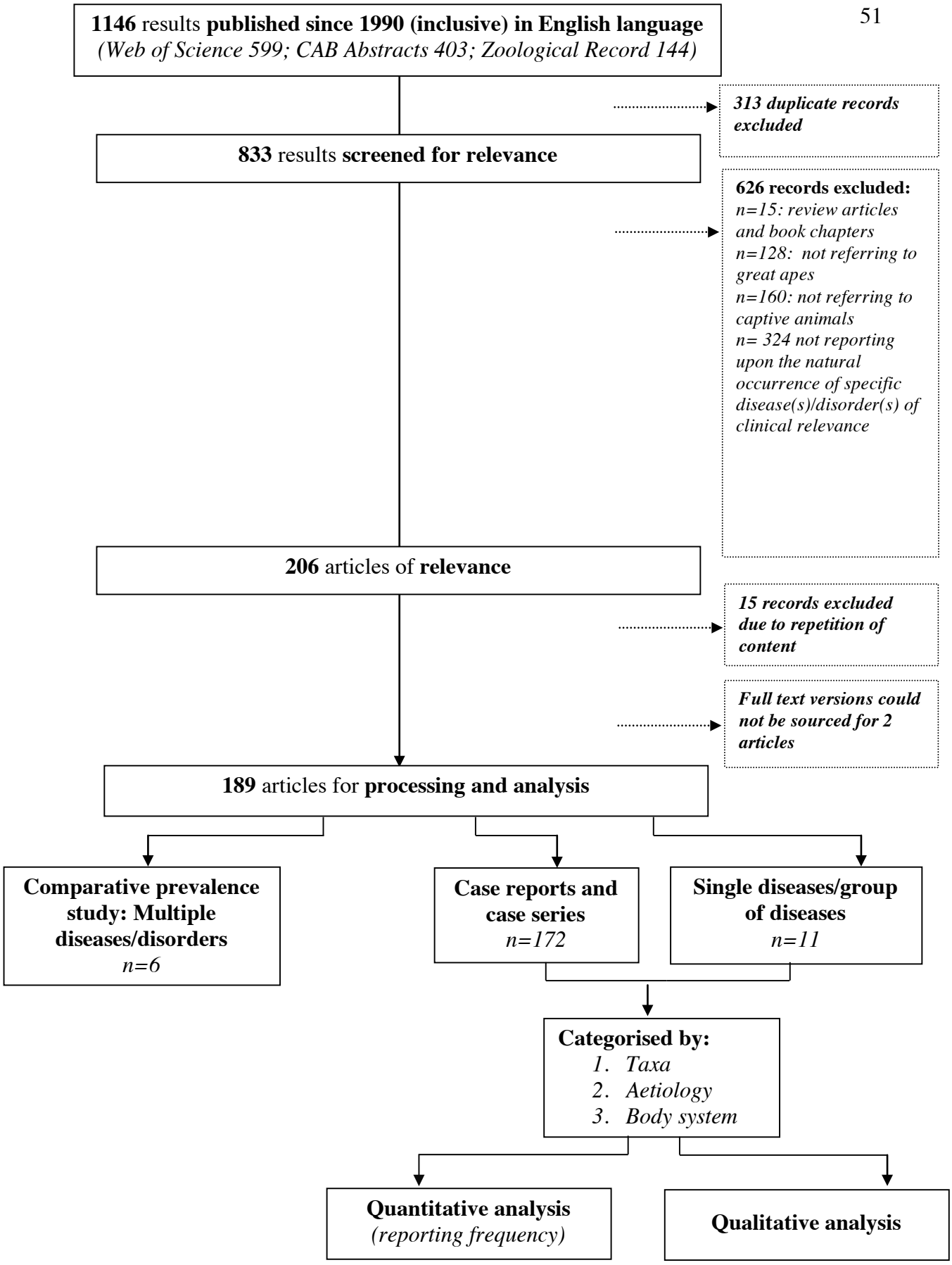


Figure 1.1 Flow chart showing sequential steps involved in reference selection and exclusion process based on the PRISMA checklist and flow diagram

1.3 Key findings and discussion

The initial search yielded 1146 results and following relevance screening, 189 full text articles remained (see Figure 1.1). The literature search revealed a variety of reference types: journal articles, conference proceedings, meeting papers and correspondence pieces. A total of 183 (97%) of the articles were classified as case reports, case series and single disease frequency studies. The remaining six papers were morbidity and mortality studies, which reported upon the comparative frequency of occurrence of various diseases and disorders within a population.

1.3.1 Case reports, case series and single disease frequency studies

The absolute number and percentage of papers identified for each taxon are displayed in Table 1.1. Chimpanzees (40%) and gorillas (35%) received greater literature coverage than orangutans (19%) and especially bonobos (2%). This distribution may be related to the relative numbers of each species kept in zoos and also due to the involvement of chimpanzees in biomedical and behavioural research (articles relating to which were included in this review).

Table 1.1 Amount of literature coverage afforded to each taxon, displayed as absolute number and percentage of total (n=183), ordered by descending proportional coverage

	Number	**Percentage of total**
Chimpanzees	73	40%
Gorillas	64	35%
Orangutans	35	19%
Two or three taxa	5	3%
Bonobos	4	2%
All four taxa	2	1%

1.3.1.1 Aetiology:

A total of 174 (95%) of the 183 papers could be categorised by the aetiology of the primary condition being reported (Table 1.2). The three aetiologies which received most literature coverage were infectious (39%), idiopathic (17%) and neoplastic disorders (9%).

Table 1.2 Amount of literature coverage afforded to each aetiology, displayed as absolute number and percentage of total (n=174), ordered by descending coverage

	Number	**Percentage of total**
Infectious	62	36%
Idiopathic	30	17%
Neoplastic	15	9%
Degenerative	11	6%
Vascular	11	6%
Behavioural/psychological	8	5%
Husbandry related	9	5%
Trauma/accidental death	7	4%
Congenital/hereditary	6	3%
Immune mediated	5	3%
Metabolic	6	3%
Miscellaneous	4	2%

Infectious diseases, especially of the gastrointestinal and upper respiratory tract, were reported more commonly in the literature from 1990-2014 than disorders of any other aetiology. This is despite the fact that infection has been stated elsewhere as becoming less important over recent years (Varki et al. 2009). The papers that reported upon infectious disease were further categorised by causal agent (Table 1.3).

Table 1.3 Articles relating to infectious diseases, sub-categorised by causal agent, displayed as percentage of total (n=62), ordered by descending coverage

	Percentage of total
Bacterial	45%
Parasitic	21%
Viral	15%
Mixed	8%
Undetermined	6%
Fungal	5%

Bacterial infection was often reported in association with disorders of the gastrointestinal system such as enteritis, colitis and the clinical presentation of diarrhoea. Reported pathogenic agents include *Salmonella* spp., *Escherichia coli* and *Campylobacter* spp. (Beutin et al. 1996; Paixão et al. 2014; Pazzaglia et al. 1994). The most commonly cited gastrointestinal disorder of bacterial aetiology was shigellosis which was also implicated in cases of reactive arthritis (Banish et al. 1990; Raphael et al. 1995; Vielgrader et al. 2005). Bacterial infections were also commonly implicated in respiratory disorders which were particularly frequently reported in orangutans (Cambre et al. 1995; Lewis et al. 2001; Steinmetz et al. 2007; Zimmermann et al. 2011). *Balantidium coli* and *Balamuthia mandrillaris* were the most frequently cited parasitic infections. Viral diseases reported, amongst others, included cases of fatal viral myocarditis

(Jones et al. 2011; Miyagi et al. 1999; Nielsen et al. 2012; Yeo et al. 2013;) and Herpes virus infection (Kik et al. 2005; Sakulwira et al. 2004). *Respiratory Syncytial Virus* (RSV), often in association with concurrent *Streptococcus pneumoniae* infection, was reported to cause severe, often fatal, bronchopneumonia (Clarke et al. 1994; Szentiks et al. 2009; Unwin et al. 2013). Fungal infections including coccidioidomycosis (Herrin et al. 2005; Hoffman et al. 2007;) and a suspected case of dermatophilosis (Brack et al. 1997) were also reported. Incidents of human-to-great ape zoonotic disease transmission reported in the literature included cases of respiratory disease due to RSV, *Streptococcus pneumoniae* and whooping cough, and cases of Coxsackie B3 virus, *Entamoeba histolytica* and Varicella virus infection (Cook et al. 2010; Gustavsson et al. 1990; Mbaya & Nwosu 2005; Nielsen et al. 2012; Szentiks et al. 2009; Unwin et al. 2013).

The majority of those articles classified as being on the topic of idiopathic disorders were reporting on the occurrence of cardiovascular disease. Reports identified suggest that disorders of the cardiovascular system are associated with high rates of mortality in at least three (chimpanzees, gorillas and orangutans) of the four great ape taxa. However, there is very little information presented in the literature about the epidemiology, diagnosis and treatment of great ape cardiovascular disease, suggesting that current understanding remains poor and there is a critical need for further research in this area. Other reported idiopathic conditions were varied, and included epilepsy, appendicitis, hyperthyroidism and diverticulitis (D'Agostino et al. 2007; Gerlach et al. 2011; McLachlan et al. 2011; Murray et al. 2000).

The gastrointestinal system (Chiu & Bodley 2010; Schauer et al. 1994) and female reproductive tract (Cook et al. 2010; Hunter & Agnew 2011; Silva et al. 2006; Stringer et al. 2010) were the body systems most commonly reported to be affected by neoplastic disorders (Table 1.4).

Table 1.4 Neoplastic disorders reported in the literature (with references), displayed by taxa, listed alphabetically

	Neoplastic disorder reported
Chimpanzee	Gastrointestinal stromal tumour (Saturday et al. 2005)
	Gingival mass (Sasseville et al. 2013)
	Hepatocellular carcinoma and myelolipoma (Porter et al. 2004)
	Maxillary sarcoma (Fujisawa et al. 2014)
	Nevus lipomatosus cutaneus superficialis (Klopfleisch et al. 2007)
	Renal carcinoma (Greenwood et al. 1995)
	Uterine leiomyoma (Silva et al. 2006)
Gorilla	Acute lymphocytic leukaemia (Barrie et al. 1999)
	Choriocarcinoma (Cook et al. 2010)
	Inoperable obstetric cancer (Hunter & Agnew 2011)
	Intracranial tumour gorilla (possible lymphoma) (Meehan et al. 1995)
	Leydigocytoma and a large cell lung carcinoma (Paixão et al. 2014)
	Metastatic pancreatic islet cell carcinoma (Chiu & Bodley 2010)
	Primary hyperparathyroidism, presumed adenoma (Hooper 2011)
	Prolactin secreting pituitary adenoma (Chatfield et al. 2006)
	Squamous cell carcinoma of the skin (Fernandez Bellon et al. 2003)
	Uterine adenocarcinoma and squamous cell carcinoma of vagina, cervix and uterus (Stringer et al. 2010)
Orangutan	Malignant gastric rhabdoid tumour (Schauer et al. 1994)

1.3.1.2 Body System:

A total of 177 (97%) of the 183 papers could be classified by body system category (Table 1.5). The body systems most commonly reported on were: cardiovascular (18%), generalised/multi-system (18%), gastrointestinal (12%) and respiratory (11%).

Table 1.5 Amount of literature coverage afforded to each body system, displayed as absolute number and percentage of total (n=177), ordered by descending proportional coverage

	Number	**Percentage of total**
Cardiovascular	31	18%
Generalised/multi-system	31	18%
Gastrointestinal	21	12%
Respiratory	20	11%
Musculoskeletal	14	8%
Neurological	12	7%
Behavioural	10	6%
Endocrine	8	5%
Reproductive	9	5%
Ophthalmic	6	3%
Skin/integumentary	6	3%
Dental	3	2%
Renal	2	1%
Urinary (lower)	2	1%
Haematopoietic/lymphatic	1	<1%
Hepatobiliary	1	<1%

Cardiovascular: Myocardial fibrosis of unknown cause (often referred to as fibrosing cardiomyopathy) was reported among gorillas, chimpanzees and orangutans and was associated with sudden death, peri-anesthetic death, congestive heart failure and

cerebrovascular infarction (Chiu & Bodley 2010; Lammey, Lee, et al. 2008; Miller et al. 1999; Munson & Montali 1990; Schulman et al. 1995). Other cardiomyopathies reported include dilated cardiomyopathy (DCM) (Slaffer & Allchurch 1995; Sleeper et al. 2005) and arrhythmogenic-right ventricular cardiomyopathy (ARVC) (Tong et al. 2013). Several reports of aortic aneurysm/dissection in male gorillas (Allchurch 1993; Kenny et al. 1994) and cardiac arrhythmias, particularly among male chimpanzees, also featured (Doane et al. 2006; Lammey et al. 2011; Sleeper et al. 2014). Hypertension was reported to affect gorillas and chimpanzees and has been shown to correlate with age and obesity and to increase mortality risk (Ely et al. 2011; Ely et al. 2013; Miller et al. 1999). Other reports included congenital heart disease (Greenberg et al. 1999; Trupkiewicz et al. 1995), fatal myocarditis (Miyagi et al. 1999; Nielsen et al. 2012; Reddacliff et al. 1997; Yeo et al. 2013), a case of coronary artery disease in a gorilla (Scott et al. 1995) and three cases of cerebrovascular accident (stroke) in female chimpanzees (Jean et al. 2012).

Generalized/multi-system: This category consisted largely of infectious disorders. Non-infectious conditions in this category included cases of idiopathic hypocalcaemia, Reyes-like syndrome (encephalopathy and hepatopathy, occurs following recovery from prior viral infection), systemic anaphylaxis, protein deficiency, clinically significant chromosomal abnormalities and intra-abdominal abscesses/cysts (Blampied et al. 1992; Bradford et al. 2013; Chatfield et al. 2012; Hayman et al. 2010; Lear et al. 2001; Minter et al. 2012; Mundy et al. 1998; Mylniczenko 2003). Obesity (with or without concurrent metabolic syndrome) was also categorised as a generalised condition (Lintzenich & Ward 2001; Nunamaker et al. 2012; Steinetz et al. 1996; Videan et al. 2007).

Gastrointestinal: Most reported gastrointestinal diseases were infection-associated. The most commonly reported presenting sign was diarrhoea, which was occasionally

haemorrhagic (Isidoro et al. 2013; Lankester et al. 2008; Paixão et al. 2014; Pazzaglia et al. 1994) but lethargy, inappetence, anorexia, tenesmus, rectal prolapse and abdominal pain also featured (D'Agostino et al. 2007; Murray et al. 2000). Three cases of neoplasia of the gastrointestinal system (Table 1.4) and one case of paralytic ileus in an orangutan, which occurred secondarily to severe depression were also reported (Sos et al. 2007).

Respiratory: Air sacculitis was the most frequently discussed respiratory disorder, especially in orangutans (Cambre et al. 1995; Lewis et al. 2001; Rietschel 2012; Zimmermann et al. 2009; Zimmermann et al. 2011). RSV with or without concurrent infection was also identified as a common cause of fatal disease. (Clarke et al. 1994; Szentiks et al. 2009; Unwin et al. 2013). Other infectious agents reported included RSV, *Strep. pneumoniae* (see 1.3.1.1), *Bordetella pertussis* (Gustavsson et al. 1990), *Strep.anginosus* (Ihms et al. 2006), *Mycobacterium kansasii* (Alvarado 1992) and *Mycobacterium tuberculosis* (Shin et al. 1995; Singh et al. 1994). Non-infectious disorders included allergic respiratory disease and anaesthetic complications (Dumonceaux et al. 1997; Karesh et al. 2012; Kenny et al. 2003). Clinical signs included nasal discharge, coughing, tachypnoea, dyspnoea and, less commonly, facial swelling, exophthalmos and cyanosis.

Musculoskeletal: The majority of musculoskeletal disorders reported were degenerative in origin (Aldridge 2005; Carter 1992; Videan et al. 2011; Wolfla & Puckett 2004). Rickets was reported in a gorilla, an orangutan and a group of chimpanzees (Bellisari et al. 2004; Chai et al. 2003; Junge et al. 2000).

Neurological: Four great apes (one orangutan; three gorillas) displayed non-specific clinical signs progressing to ataxia, disorientation, and death caused by the amoeba species *Ballamuthia mandrillaris* (Canfield et al. 1997; Mätz-Rensing et al. 2011; Rideout et al. 1997). Encephalopathy was also the cause of death in a case of septicaemia due to *Aeromonas*

hydrophilia infection in a gorilla (Harrison et al. 2001). Non-infectious neurological conditions reported include inflammatory polyradiculoneuropathy, cerebral calcinosis, vascular mineralisation and acute transverse myelitis in chimpanzees (Alford & Satterfield 1995; Connor-Stroud et al. 2014; Miyabe-Nishiwaki et al. 2010; Zoller et al. 2005); intracranial mass, hydrocephalus and age-related pallido-nigral degeneration in gorillas (Márquez et al. 2008; Meehan et al. 1995); demyelinating polyneuropathy in an orangutan (Cossaboon et al. 2008); and epilepsy in bonobos (Gerlach et al. 2011).

Behavioural: One observational study of zoo housed chimpanzees concluded that abnormal behaviour is endemic in the population (Birkett & Newton-Fisher 2011). The paper reported that all 40 animals displayed at least two abnormal behaviours including coprophagy, stereotypic grooming, genital touching, rocking, regurgitation, hair plucking and self-injury. Regurgitation/re-ingestion syndrome was also reported in gorillas (Hill 2009; Lukas et al. 1999) chimpanzees (Struck et al. 2007) and orangutans (Cassella et al. 2012). Self-injurious behaviour was identified in a gorilla (Espinosa-Avilés et al. 2004), a chimpanzee (Bourgeois et al. 2007) and a bonobo (Prosen & Bell 2001). Mood and anxiety disorders were also described in chimpanzees (Bradshaw et al. 2008; Ferdowsian et al. 2011).

Other body systems: Disorders of the integument, reproductive and genitourinary tracts, endocrine, hepatobiliary and hematopoietic/lymphatic systems as well as ophthalmic and dental conditions each constituted five percent or less of the articles identified.

Whilst these figures relating to the frequency of reporting, are informative, it must be remembered that amount of literature coverage a disorder receives might be influenced by several factors, for example: the veterinary knowledge and diagnostic capabilities available at the time; the level of allocated funding; the specific clinical and academic interests of the author; or simply the author's perceived importance of the condition. Perhaps almost ironically,

an author's perception of a condition's importance can in turn be influenced by the amount of literature coverage it receives. Case reports are an invaluable resource but they give very little perspective on the significance of a condition at a population level. An appreciation of this latter point requires in depth epidemiological study and the calculation of comparative prevalence of various diseases and disorders. This was the purpose of just six of the papers identified by the literature search (see Table 1.6).

1.3.2 Morbidity and mortality studies

Six (3%) of the 189 papers identified consisted of reviews of the various causes of morbidity and mortality among captive chimpanzees, gorillas and orangutans. The main findings of each are summarised in Table 1.6. Three of the six prevalence reviews were single-centre studies (Lammey, Baskin, et al. 2008; Munson & Montali 1990; Nunamaker et al. 2012). The three remaining studies reviewed morbidity and mortality across more than one institution (Hewitt 2005; Meehan & Lowenstine 1994; Varki et al. 2009). This allowed for a larger number of animals to be studied, arguably deeming the findings of these latter studies more representative of the wider population. Five of the six were retrospective reviews (Hewitt 2005; Meehan & Lowenstine 1994; Munson & Montali 1990; Varki et al. 2009) whereby the data analysed was in fact information which had been collected for another purpose. The researchers therefore had very little control over the accuracy and completeness of the dataset, which might negatively impact upon the reliability of the findings. In contrast, the screening of 16 geriatric female chimpanzees for the presence of chronic and age related disease (Nunamaker et al. 2012) was a prospective study and therefore had the advantage that data collection methods were specifically designed for purpose. The results of this study might therefore be considered more reliable, although the potential for observer bias (bias introduced due to the investigator's

prior knowledge of the hypothesis under investigation of the individual's exposure/disease status) (Barratt & Kirwan 2009) remains. All six studies reported the frequency of occurrence of various conditions but were only able to speculate about underlying causes and risk factors.

Table 1.6 Summary of key components of the six morbidity/mortality reviews identified by the literature search

Author and year	Period of study	Study type	Study population	Main Causes of Morbidity/Mortality
Meehan and Lowenstine 1994	1980-1994	Retrospective, observational, longitudinal, multi-center (unknown no.); review of post-mortem records	Gorillas (n=74); all ages/genders; SSP population	Infants: trauma (60%) Adults (<30y): gastrointestinal (36%); cardiovascular (32%) Elderly (>30y): cardiovascular (53%)
Varki et al. 2009	1966-1991 1992-2008	Retrospective, observational, longitudinal, multi-center (2); review of post-mortem records	Chimpanzees (n=58); adult (>10y); Yerkes National Primate Research Center; Primate Foundation of Arizona	1966-1991: enterocolitis (16%); heart disease (11%); meningitis (11%); pneumonia (5%); renal disease (5%); trauma (3%); miscellaneous (50%) 1992-2008: heart disease (36%); renal disease (16%); trauma (12%); miscellaneous (36%)
Nunamaker et al. 2012	2009	Prospective, observational, cross-sectional single center; incidence of disease at routine health assessment	Chimpanzees (n=16); aged >35y; females; Alamogordo Primate Facility	Cardiovascular disease (81%); metabolic syndrome (44%); renal disease (31%)
Munson and Montali 1990	Unknown	Retrospective, observational, longitudinal, single center; review of pathology and medical records	Gorillas, orangutans and chimpanzees (n=unknown); National Zoological Park, Washington	Orangutans: myocardial fibrosis; gastrointestinal disease; perinatal infections Gorillas: arthritis; gastrointestinal disease; infertility; Chimpanzee: myocardial fibrosis
Lammey, Baskin, et al. 2008	2001-2006	Retrospective, observational, longitudinal, single center; review of post-mortem records	Chimpanzees (n=36); aged 10-40y; Alamogordo Primate Facility	Sudden cardiac death (36%); renal failure (25%); trauma and septicemia (each 23%); anesthetic complications and neoplasia (each 15%)
Hewitt 2005	1896-2005	Retrospective, observational, longitudinal, multi-center (9); review of post-mortem records	Gorillas (n=109); all ages; UK and Ireland population (various zoological collections)	Respiratory (27%); multi-system (26%); gastrointestinal (15%) (cardiovascular disease: <9%)

Inclusion criteria were not always stated, making it difficult to ascertain the degree of selection bias present. In two of the multi-centre studies (Hewitt 2005; Meehan & Lowenstine 1994), the investigators were reliant on various institutions submitting data to the study. This self-selection process has the potential to introduce bias but in retrospective studies which involve voluntary participation, is largely unavoidable.

In five of the six studies (Hewitt 2005; Lammey, Baskin, et al. 2008; Meehan & Lowenstine 1994; Munson & Montali 1990; Varki et al. 2009), the data collected consisted of medical records or pathology reports written by various clinicians and pathologists at the time of an animal's illness or death. In one study (Hewitt 2005) reported morbidity and mortality events dating as far back as 1896, when veterinary diagnostic capabilities were limited.

Three of the six studies were included animals kept in primate research (laboratory) facilities, the management of which differs greatly from that in zoos. In view of this fact, as well as the issues associated with small sample populations, time-span and single-centre studies already discussed, the findings of many of the six studies may not be representative of the wider population, nor provide an accurate and up to date representation of the current situation in zoos.

1.4 Literature review: limitations

The validity of the conclusions depends heavily on the reliability of the initial literature search. Every effort was made to make the search as exhaustive as possible though the use of synonyms and MeSH terms. The search strategy used within this review returned a total of 1146 initial results, which were considered to constitute a representative proportion of the entire literature body. Less than one third (n=313) of the 1146 results were duplicates suggesting that there is a relatively low degree of overlap of coverage by the databases accessed. Some relevant papers were not identified by the literature search (Chai et al. 2009; Lung et al. 2004; Pollock et al. 2008). This might have been due to words, such as captive or zoo, not being included in the keywords provided by authors or at the point of database indexing. This highlights the importance of the application of accurate and representative keywords by journal authors and editors to facilitate such search and review processes. These omissions might however simply reflect the inherent limitations of any literature search, and the difficulties associated with striking the balance between maximising sensitivity whilst retaining specificity.

For case report/case series or single diseases category definitions were described and closely adhered to but some degree of subjectivity and ambiguity was still encountered. An example of this is regurgitation/re-ingestion, categorised in this review as a behavioural disorder. However, since diet and environmental conditions have been suggested as underlying causes, it might have been categorised as husbandry-related, illustrating how easily categorisation can influence results.

Differences in the approach to categorisation might also have been responsible for the variation in findings between the two gorilla comparative mortality studies identified by the search: Meehan and Lowenstine (1994) found that cardiovascular disease was responsible for 32% of adult and 41% of aged gorilla deaths. However, Hewitt (2005) identified it as the cause of death

in less than 9% of the animals. If this discrepancy were genuine, it might suggest that there are differences in the cardiac risk factors (such as genetics, diet, husbandry) to which the North American and UK/Irish populations are exposed. Upon closer examination, however, several animals included in the latter study were diagnosed as having cardiovascular and another concurrent disease on post-mortem. These deaths were categorised as being multi-system in origin, which might have led to underrepresentation of the importance of cardiovascular disease as a cause of mortality within this population.

Categorisation by aetiology does not account for disorders or events which occur due to a more complicated, multi-factorial pathophysiology. Examples of such are anaesthesia-related complications and infection-associated disorders. In the latter case, the pathogen may not be the primary or sole cause; the infection might be a complicating factor in another disease process or may be occurring secondarily to underlying reasons such as immunosuppression, poor husbandry or environmental conditions. In human medicine, many of the issues highlighted here are overcome by the implementation of the International Classification of Diseases system, ICD-10 (WHO 2010). This might in fact be a more appropriate categorisation model for morbidity and mortality reviews in veterinary medicine to utilise in the future.

1.5 Literature review: conclusions

This was the first review of the great ape literature published since 1990 and the first ever systematic literature review of great ape morbidity and mortality. It showed that the body system receiving the greatest literature attention during the period under study was the cardiovascular system. Whilst high rates of heart disease associated morbidity and mortality have been reported, the epidemiology and aetiopathogenesis of the conditions described remain unknown, suggesting a need for further research in this area.

The review also showed that there is a critical need for a robust, widespread and more up-to-date review of mortality among zoo-housed great apes. This is especially true for those housed in European collections, for which no such published or unpublished reviews have been performed to date. This latter conclusion formed the foundation for Chapter 2 of this thesis.

CHAPTER 2:

A RETROSPECTIVE REVIEW OF MORTALITY AMONG EUROPEAN AND NORTH AMERICAN ZOO-HOUSED GREAT APES

2.1 Introduction

A systematic review of the literature relating to the diseases and disorders affecting captive great apes was carried out (Strong et al. 2016; Chapter 1). The review identified a lack of recent data especially for the European zoo-housed great population and highlighted a critical need for a robust, widespread, and up to date review of mortality within this population. This conclusion formed the basis for this chapter of this thesis.

The aims and objectives of the study were as follows:

1. To identify and quantify the significance of the main causes of mortality for each:
    a. Taxa of great ape taxa housed in zoological collections across Europe
    b. Age group
2. To be a multi-centre study with excellent participation rates to make the findings representative of the European zoo-housed great ape population as a whole
3. To focus on a relatively recent time-period to provide an accurate and up to date representation of the current situation within this population
4. To utilise a systematic and repeatable methodology which can be used to draw comparisons: between species, between other (including wild) populations of the same species and within the same population over time

The findings of such a study would be informative not only for the practicing clinician, but might also serve as a model for future mortality studies of its type. It would lay the foundations for the ongoing monitoring of patterns of mortality within this and other populations, the key ambition of which being to inform future management practice and policy, and ultimately to enhance of the health and welfare of the animals concerned.

2.2 Materials and methods

2.2.1 Study population

For chimpanzees (*Pan troglodytes*), western lowland gorillas (*Gorilla gorilla gorilla*), and Bornean and Sumatran orangutans (*Pongo pygmaeus, Pongo Abelii)*, the study population was defined as any animal which:

a) Died during the period 1st January 2004 to 31st December 2014

b) Was housed in a European Association of Zoos and Aquaria (EAZA) member zoo and

c) At the time of death, was housed within a European zoological collection

For bonobos, for which the captive population is comparatively smaller, the study population criteria were extended to also include those animals managed under the Association of Zoos and Aquaria (AZA) Species Survival Plan (SSP), and housed in North American collections.

2.2.2 Raw data collection

Information about the study population and a report of all deaths during the study period were generated from studbook data using the Single Population Analysis and Record Keeping Software (SPARKS) (Species360, 2005). Further information (clinical notes, animal records and post-mortem examination reports) relating to each of the deaths was requested from the holding collection. The institutions were invited to provide this information in any available format (handwritten notes, electronic records) and to send it via email or post. Each institution was contacted on at least two separate occasions and non-responses also followed up by the relevant species coordinator to maximise participation rates.

2.2.3 Data processing

Where necessary, the information was translated into English. The raw data was reviewed and key information extracted. The data fields selected were developed from those used in previous similar studies (Gamble et al. 2004; Hewitt 2005; Meehan & Lowenstine 1994). For each animal, the data fields included: animal identification information (regional studbook number; name), zoological collection (at the time of death), sex (male; female; undetermined/unrecorded), date of birth and death. Some animals were wild-born and so their exact dates of birth are unknown. In these instances, if only a month and year was provided for the date of birth, the date was assumed to be the first day of that month. Where only a year was provided, the date was assumed to be the first day in July of that year. The potential for this assumption to introduce bias into the dataset was explored for each of the taxa by studying the effect that moving the birth date to the minimum and maximum possible would have had on the subsequent categorisation by age at death (see Section 2.3.1.1). The age at death was calculated from the dates of birth and death. The following information was also collected and added to the spreadsheet: Was macroscopic post-mortem examination data available? (yes/no); was histopathology data available? (yes/no); what was the main pathological lesion/condition associated with death? (free text).

Each animal was categorised by their age at the time of death. To ensure consistency across the taxa, all animals less than one year of age were classified according to the definitions used for human infant mortality: foetal (stillborn/premature/abortion; had never breathed), perinatal/early-neonate (breathed, but died aged 0-7 days), and infant (8-364 days) (World Health Organisation 2006). Animals one year of age or older were grouped into species specific, discrete age categories (Alberts et al. 2013; Furuichi et al. 1998; Galdikas 1981; Gamble et al. 2004; Meehan & Lowenstine 1994; Rowe 1996) (Table 2.1).

Table 2.1 Upper and lower limits in years (y) used for each age category. For gorillas and orangutans, the age category limits differed for male (M) and female (F) subjects

	Bonobo	**Chimpanzee**	**Gorilla**	**Orangutan**
Juvenile	1-7y	1-10y	1-7y (F); 1-10y (M)	1-8y
Adolescent/sub-adult	7-15y	10-20y	7-15y (F); 10-15y (M)	8-15y (F); 8-18y (M)
Adult	15-35y	20-35y	15-35y	15-35y (F); 18-35y (M)
Aged/elderly	>35y	>35y	>35y	>35y

Deaths were categorised by cause as identified in the post-mortem information provided. Cause of death categories (see Appendix 5) were developed based upon those in the International Classification of Diseases (ICD) system, which is widely utilised in human medicine and epidemiology (World Health Organisation 2010). In cases of euthanasia, the death was categorised according to the main pathological lesion or illness which was reported to be responsible for the decision to euthanase. If the reason for this decision or the animal's ultimate deterioration in health was not made clear in the information provided, the death was categorised as miscellaneous. In cases of anaesthetic death, if a cause was identified in the post-mortem report (for example decompensated cardiac or respiratory disease), the death was categorised accordingly. In all other instances, anaesthetic deaths were categorised as being due to external causes. 'External causes' also referred to deaths caused by trauma, injury or starvation and accidental deaths (Table 2.7).

Data cleaning was performed: the inputted data was checked for errors prior to analysis.

The main causes of death for each taxa and age category were identified and are presented in Section 2.3.

2.2.4 Data analysis

Statistical analyses were carried out using Microsoft Excel (Microsoft 2010) and GraphPad Prism 7 for MacOS X (2016). Sex variation in age at death was assessed using log-rank (Mantel-Cox) analysis and the Kaplan-Meier method. Confidence intervals were set at 95% and p-values of <0.05 were considered significant. Data were assessed for normality using a D'Agostino & Pearson omnibus test. Normally distributed data as expressed as mean and standard deviation, and non-normally distributed data as median (min; max) unless otherwise stated.

2.3 Results

2.3.1 Study population

At the end of the study period (on 31st December 2014) there were 191 bonobos, 1037 chimpanzees, 421 gorillas and 352 orangutans housed in EAZA member zoos in Europe (Becker 2015; Carlsen & Jongh 2015; Pereboom et al. 2015; Wilms & Bender 2015).

A total of 681 great apes were reported to have died during the study period (Appendix 6). Basic information relating to the animals' identification, zoological collection, dates of birth (sometimes estimated) and death were available in all cases. The animals were housed in 136 different collections across 26 countries. More detailed post-mortem information for at least one animal was provided by 103 of these collections, giving a participation rate of 76%.

2.3.1.1 Effects of estimated birth dates

The exact date of birth was unknown and estimated birth dates used for 125 of the animals undergoing cause of death analysis (Table 2.2).

There were 28 animals for which only a month of birth was provided; these were given a birth date of the first day of that month. For 97 animals, only a year of birth was given; these animals were assigned an estimated birth date of 1st July of that year. Moving the estimated date of birth to the earliest and latest possible dates of birth for each of these animals would only have resulted in the re-categorisation of only six (n=6/411; 1%) animals, meaning that the amount of bias introduced into the data by date of birth estimation was negligible.

Table 2.2 Data relating to those animals for which an exact date of birth (DOB) was not provided and for which estimated birth dates were used, including the effect of estimation on age categories assigned

		Bonobo	**Chimpanzee**	**Gorilla**	**Orangutan**
Number of animals undergoing cause of death analysis		38	211	102	60
Exact DOB unknown	Only month provided, *n*	*0*	*17*	*11*	*0*
	Only year provided, *n*	*9*	*48*	*23*	*17*
	Total, n	**9**	**65**	**34**	**17**
Re-categorisation	Number, *n*	0	2	3	1
	Effect	None	Adult » Aged	2 Aged » Adult 1 Adult » Aged	1 Adult » Aged

2.3.2 Post-mortem examination and data quality

The quality and extent of the post-mortem data provided by collections ranged from a single line of information, to a complete and comprehensive report detailing the normal and abnormal findings in each organ/body system. In some cases, basic information such as animal identification, date of birth, date of death or age was missing from the post-mortem report, however, cross referencing with studbook data allowed the relevant information to be obtained.

It was evident from the reports, that the extent of post-mortem examination routinely performed varied significantly between collections, ranging from basic external visual inspection by zoo curatorial +/- veterinary staff to a full detailed examination by a board certified medical or veterinary pathologist at an academic or diagnostic facility. The organs/samples examined histologically also varied: some institutions carried out histology on samples from every organ

as a matter of course, with others only doing so on those organs which appeared grossly abnormal or which were clinically suspected to be diseased.

In some cases, the reason for limited detail being provided in the report was given. Most commonly cited was poor carcass condition, either due to autolysis or severe post-mortem damage by conspecifics. Carcass damage was particularly common among chimpanzees and in infants, which were often carried by the dam for prolonged periods of time before being acquired/presented for examination. In a small number of cases, however, detailed post-mortem examinations were not carried out despite no reports of carcass damage or autolysis. The reasons given were that "the cause of death was known" or that "the animal was elderly".

2.3.3 Foetal deaths

Stillbirths and abortions accounted for 10% (n=67/681) of the deaths in this study (Table 2.3). For 58% (n=39/67) of these animals, the death was merely reported by the collection as being a stillbirth or abortion, and little or no further information was provided. However, a report detailing the findings of a macroscopic examination was available for 42% (n=28/67) of the animals, and a histopathology report for 22% (n=15/67). These figures did however did vary greatly depending on the taxa, for example: macroscopic examination reports were provided for 90% (n=9/10) of orangutans but only 26% (n=9/35) of chimpanzees.

Table 2.3 Data relating to foetal deaths across all four taxa

		Bonobo	**Chimpanzee**	**Gorilla**	**Orangutan**	**TOTAL**
Number of deaths, *n*		**47**	**370**	**151**	**113**	**681**
Foetal deaths, *n (% of total)*		8 (17%)	35 (9%)	14 (9%)	10 (9%)	**67 (10%)**
Data availability	Macroscopic examination report, *n (%)*	5 (63%)	9 (26%)	5 (36%)	9 (90%)	**28 (42%)**
	Histopathology report, *n (%)*	3 (38%)	5 (14%)	3 (21%)	4 (40%)	**15 (22%)**
Cause	Abortion, *n (%)*	4 (50%)	7 (20%)	4 (29%)	0 (0%)	**15 (22%)**
	Premature birth, *n (%)*	0 (0%)	1 (3%)	0 (0%)	1 (10%)	**2 (3%)**
	Dystocia associated, *n (%)*	1 (12.5%)	7 (20%)	2 (14%)	4 (40%)	**14 (21%)**
	Stillbirth unknown/other, *n (%)*	3 (37.5%)	20 (57%)	8 (57%)	5 (50%)	**36 (54%)**

Abortions and dystocia-associated deaths were responsible for 22% (n=15/67) and 21% (n=14/67) of all foetal deaths respectively. Deaths associated with premature births were reported in only two (3%) animals. The remaining and majority of foetal deaths (n=36/67, 54%) were stillbirths of other (intrauterine asphyxia; n=1) or unknown cause.

Foetal deaths were excluded from age at death and cause of death analysis (Appendix 6).

2.3.4 Age at death analysis

A total of 586 animals were subject to age at death analysis. Of these, 11% (n=65/586) were perinatal; 14% (n=82/586) were infant; 12% (n=70/586) were juvenile; 12% (n=70/586) were sub-adult/adolescent; 28% (n=163/586) were adult; and 23% (n=136/586) were aged/elderly at the time of death.

Table 2.4 Data relating to age at death (d: days; y: years) across all four taxa, for male (M) female (F) and all (total) animals undergoing age at death analysis.

Taxa	*Sex*	*Number of values*	*Age at death: range*	*Age at death: average**
Bonobo	M	23	0d. to 38.55y	15.45 (1.16; 29.16)
	F	15	0d. to 61.78y	20.89 ± 20.95
	T	**39**	**0d. to 61.78y**	**17.59 ± 16.75**
Chimpanzee	M	128	0d. to 56.48y	19.20 (1.64; 32.16)
	F	173	0d. to 59.44y	21.25 (1.75; 34.81)
	T	**321**	**0d. to 59.44y**	**18.86 (0.88; 32.90)**
Gorilla	M	53	0d. to 51.21y	21.41 (0.53; 35.86)
	F	75	0d. to 53.81y	21.87 (0.60; 37.38)
	T	**129**	**0d. to 53.81y**	**21.53 (0.54; 36.51)**
Orangutan	M	45	4d. to 57.29y	23.17 (4.42; 41.57)
	F	51	0d. to 53.22y	21.57 (4.78; 28.85)
	T	**97**	**0d. to 57.29y**	**21.80 (4.42; 37.31)**

**Average refers to the mean for parametric data and the median for non-parametric data*

The age at death data presented (Table 2.4) suggests that orangutans exhibit the greatest average longevity of all the taxa, whereas bonobos exhibit the least. This trend for reduced life expectancy in bonobos is also observed when age at death data is plotted onto a Kaplan-Meier

curve (Figure 2.1). Whilst longevity appears to follow a similar pattern across the taxa, the graph suggests a more rapid decline in bonobo survival above the age of 25 years. However, log-rank (Mantel-Cox) analysis showed no statistically significance in survival between the four taxa (p=0.8271).

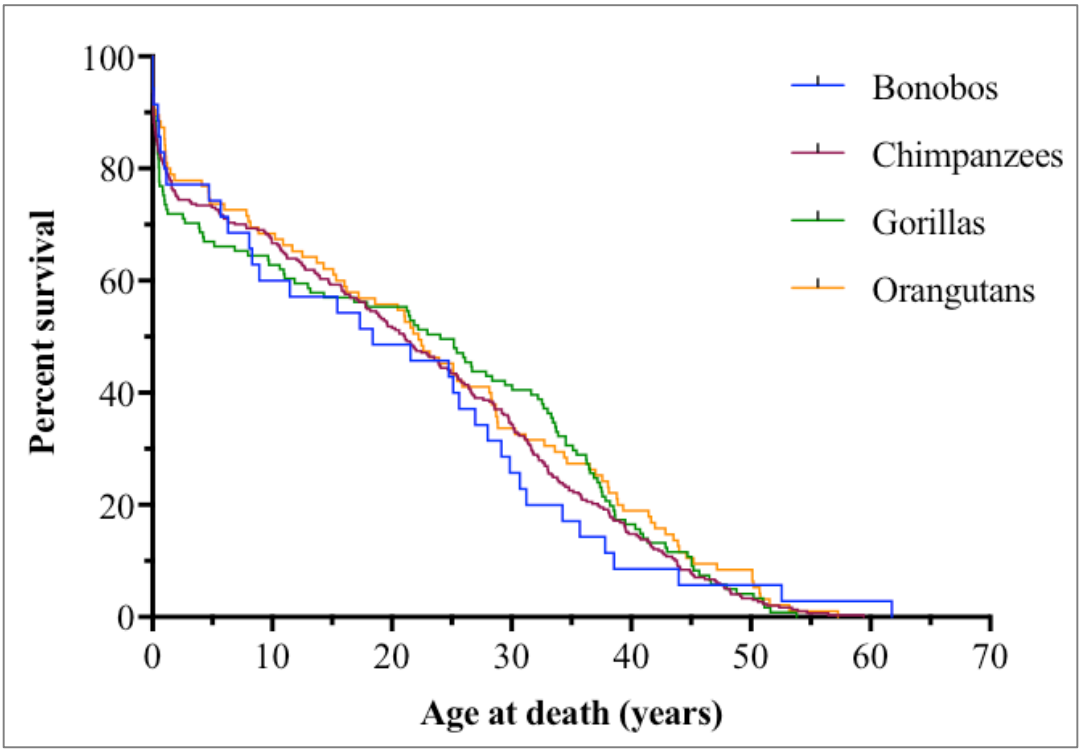


Figure 2.1 Kaplan-Meier curve showing age at death data for all four taxa

Across bonobos, chimpanzees and gorillas, the average age at death was lower for males than females (Table 2.4 and Figure 2.2). However, the reverse was true of orangutans for which both the median and maximum ages at death were lower among females. The appearance of the Kaplan-Meier curves gave the impression that these differences were most apparent later in life. However, log rank (Mantel-Cox test) analysis showed no statistically significant difference in survival between the genders overall nor for bonobos, chimpanzees or gorillas aged over 20 years. There was however, a significant difference (p=0.0149) in age at death among orangutans >20 years of age, among which female survival was reduced.

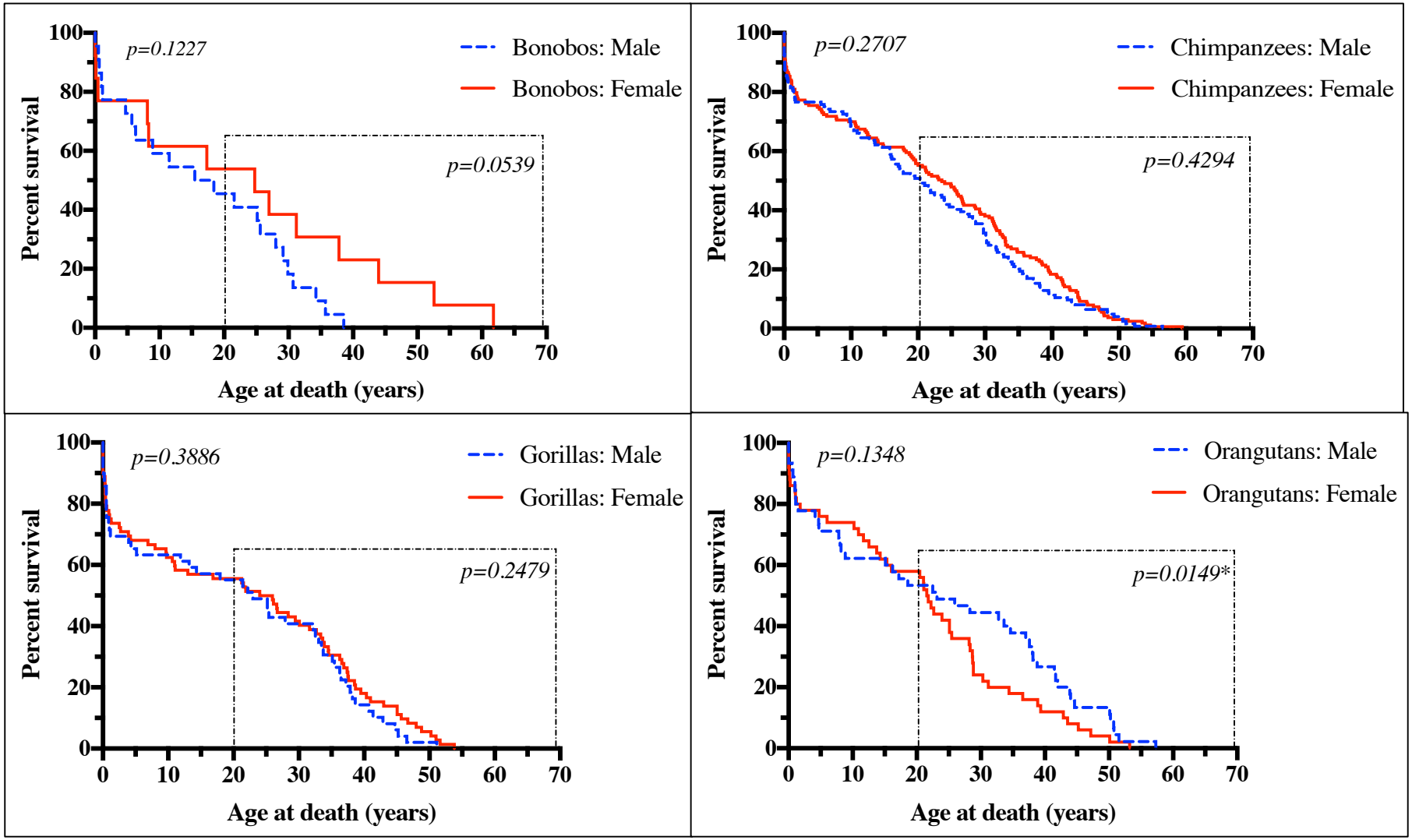


Figure 2.2 Kaplan-Meier curves comparing age at death for male and female bonobos (top left), chimpanzees (top right), gorillas (bottom left) and orangutans (bottom right). *P values displayed within the dashed lines relate to log rank (Mantel-Cox) analysis results for animals aged >20 years. *=statistically significant result (p<0.05)*

2.3.4.1 First 5-year mortality

Mortality in the first five years of life was high across the taxa (Figure 2.3). Of the gorilla (n=48/129, 37%), chimpanzee (n=104/321, 32%) and bonobo (n=13/39, 33%) deaths that occurred during this period, >80% were in animals aged <12 months (Figure 2.4a). Comparatively fewer orangutan deaths occurred in early life: 28% (n=27/97) of orangutan deaths occurred in the first 5 years of life, of which just over half (n=15/27; 56%) occurred in animals <12 months of age. The highest risk for infant mortality for the animals under study was during the perinatal period, with 44% (n=65/147) of infant deaths occurring in animals aged 0-7 days (Figure 2.4b). Among bonobos the observed peak in early life mortality persisted into the 5-10-year age interval, with animals in this category accounting for a further 13% (n=5/39) of all bonobo deaths.

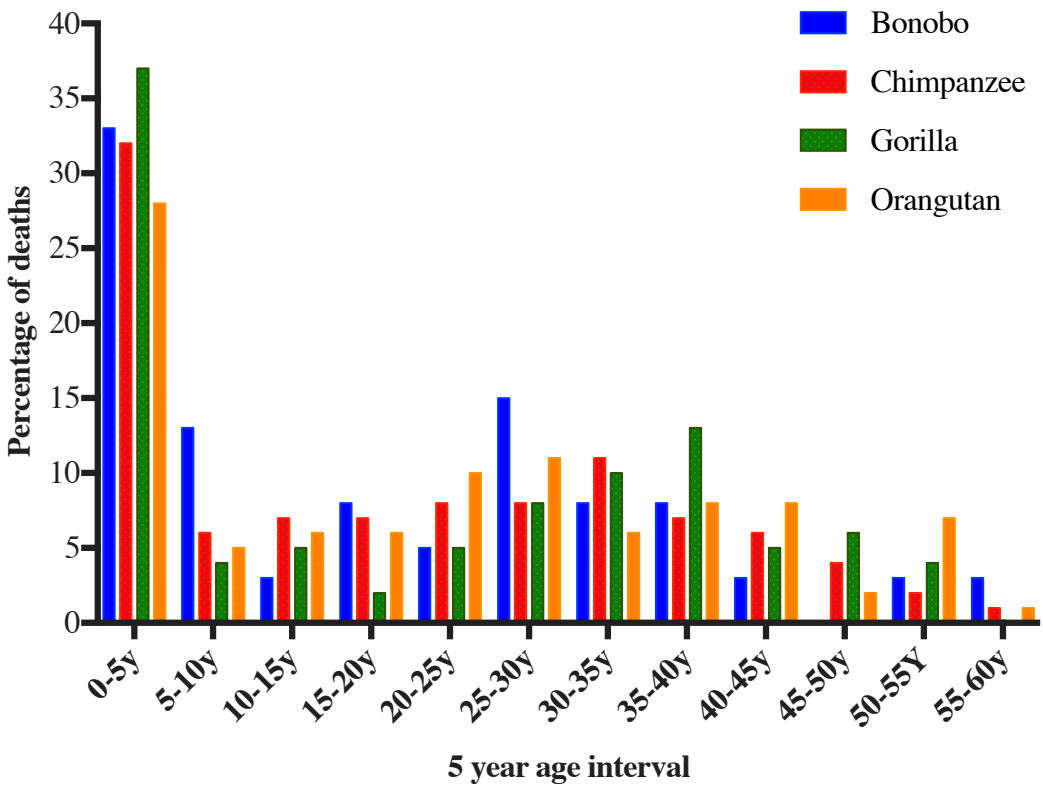


Figure 2.3 Bar chart showing the percentage of deaths occurring within each 5-year interval for all four taxa

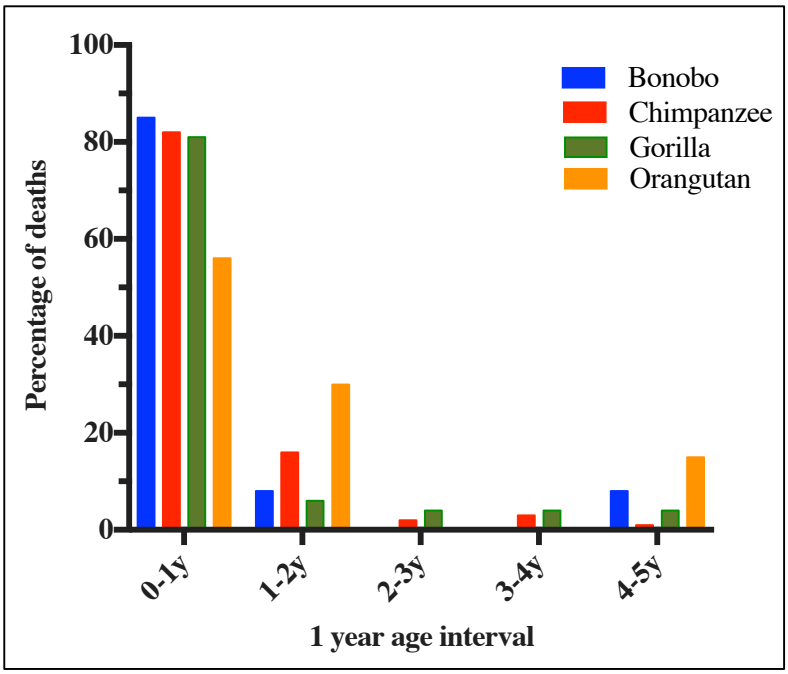


Figure 2.4a Bar chart showing the percentage of deaths occurring within each 1-year interval from 0-5 years for all taxa

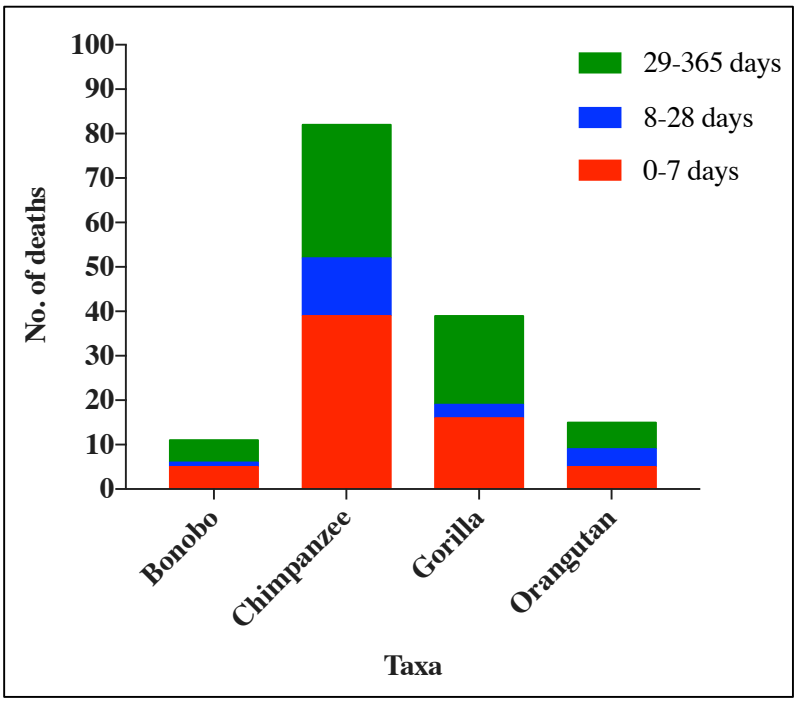


Figure 2.4b Bar chart showing the proportion of deaths occurring during each period (perinatal 0-7d.; neonatal 8-28d.; infant 29-365d.) of life

2.3.5 Cause of death analysis

Data from 411 animals were subject to cause of death analysis (Table 2.5). Those animals for which data were not provided (n=156) or for which, despite a post-mortem examination being carried out, the cause of death was unknown/undetermined (n=19) were excluded from cause of death analysis (see Appendix 6).

2.3.5.1 Overall mortality

Bonobos: The most common cause of death among bonobos was disease of the circulatory (cardiovascular) system, which was responsible for the death of over one third (n=13/38; 34%) of the animals under study. This was closely followed by respiratory disease, which was identified as the cause of 32% (n=12) of the deaths. Death due to external causes was the third most common cause of bonobo death, being associated with 16% (n=6) overall mortality.

Chimpanzees: External causes was the most frequently encountered cause of death category in chimpanzees (n=61/211; 29%), however, disease of the circulatory (n=37/211; 18%) and respiratory (n=32/211; 15%) systems were also responsible for significant overall mortality.

Gorillas: Among gorillas, it was diseases of the digestive system which were responsible for the greatest proportion (n=23/102; 23%) of deaths. Associated with equal overall mortality (n=15/102; 15%) were diseases of the circulatory system and external causes.

Orangutans: Respiratory disease was responsible for the greatest overall orangutan mortality, being the cause of death in 27% (n=16/60) of cases. Also of significance were deaths occurring due to external causes (n=12/60; 20%) and neoplastic disorders (n=7/60; 12%).

Table 2.5 Data relating to cause of death for all four taxa, displayed as total (T); percentage (%); and divided into male (M), female (F) and unknown/undetermined (U) sex.

The most frequently identified cause(s) of death for each taxa (total only) is shown in blue text; 2nd most frequent in green; third most frequent in orange

	BONOBO					CHIMPANZEE					GORILLA					ORANGUTAN				
	M	*F*	*U*	***T***	***%***	*M*	*F*	*U*	***T***	***%***	*M*	*F*	*U*	***T***	***%***	*M*	*F*	*U*	***T***	***%***
Behavioural/management	-	-	-	**-**	**-**	8	-	-	***8***	***4%***	-	-	-	**-**	**-**	-	-	-	**-**	**-**
Circulatory	9	4		***13***	***34%***	20	17	-	***37***	***18%***	13	2	-	***15***	***15%***	3	3	-	***6***	***10%***
Congenital	-	-	-	**-**	**-**	-	-	-	**-**	**-**	-	-	-	**-**	**-**	-	2	-	***2***	***3%***
Digestive	-	-	-	**-**	**-**	7	8	-	***15***	***7%***	16	7	-	***23***	***23%***	2	3	-	***5***	***8%***
Endocrine	-	-	-	**-**	**-**	-	1	-	***1***	**-**	--	1	-	***1***	***1%***	-	-	-	**-**	**-**
External causes	3	3	-	***6***	***16%***	23	30	8	***61***	***29%***	2	12	1	***15***	***15%***	4	7	1	***12***	***20%***
Genitourinary	-	1	-	***1***	***3%***	9	1	-	***10***	***5%***	-	6	-	***6***	***6%***	-	-	-	**-**	**-**
Infectious	2	-	-	***2***	***5%***	4	3	-	***7***	***3%***	3	6	-	***9***	***9%***	2	3	-	***5***	***8%***
Miscellaneous/other	-	-	-	**-**	**-**	-	5	-	***5***	***2%***	1	-	-	***1***	***1%***	-	2		***2***	***3%***
Musculoskeletal	-	-	-	**-**	**-**	1	3	-	***4***	***2%***	-	1	-	***1***	***1%***	-	-	-	**-**	**-**
Neoplastic	1	-	-	***1***	***3%***	1	4		***5***	***2%***	1	7		***8***	***8%***	4	3		***7***	***12%***
Neurological	-	1	-	***1***	***3%***	-	5	-	***5***	***2%***	2	-	-	***2***	***2%***	1	-	-	***1***	***2%***
Perinatal	1	-	1	***2***	***5%***	5	9	4	***18***	***9%***	6	4	-	***10***	***10%***	-	1	-	***1***	***2%***
Pregnancy/parturition	-	-	-	**-**	**-**	-	2	-	***2***	***1%***	-	1	-	***1***	***1%***	-	-	-	**-**	**-**
Reproductive	-	-	-	**-**	**-**	-	1	-	***1***	**-**	-	-	-	**-**	**-**	-	3	-	***3***	***5%***
Respiratory	7	5	-	***12***	***32%***	13	17	2	***32***	***15%***	8	2	-	***10***	***10%***	10	6	-	***16***	***27%***
TOTAL (n)	*23*	*14*	*1*	***38***		*91*	*106*	*14*	***211***		*52*	*49*	*1*	***102***		*26*	*33*	*1*	***60***	

2.3.5.2 Age specific mortality

A breakdown of all causes of death for each of the age categories is provided in Appendix 7.

Perinatal, infant and juvenile mortality: For those animals dying in the first seven days of life, the main causes identified across the taxa were those conditions arising in the immediate perinatal period (including birth complications, maternal rejection and starvation) and external causes (mostly trauma by dam or conspecifics). Among infants and juveniles, it was external causes and diseases of respiratory system which were responsible for the greatest number of deaths, with these categories accounting for more than two thirds of the combined mortality across the taxa. Only slight differences were observed between the taxa: Among infant bonobos, death due to external causes was not reported. Instead, diseases of the neurological system accounted for 20% (n=1/5) of the deaths and respiratory disease for the remaining 80% (n=4/5). However, death due to external causes was responsible for 75% (n=3/4) of juvenile bonobo deaths. Among gorillas, disease of the digestive system accounted for equal proportional infant mortality (n=4/17; 24%) as respiratory disease. It was also responsible for 14% (n=1/7) of juvenile gorilla deaths. One third (n=2/6) of the juvenile orangutan deaths occurred due to congenital disorders, which were not identified as a cause mortality in any other taxa.

Sub-adult, adult and aged/elderly mortality: The greatest variation in cause of death between the taxa was observed among sub-adult and adolescent animals. In this age category, the most frequently identified causes were: respiratory disease (n=3/4; 75%) among bonobos; external causes (n=8/28; 29%) among chimpanzees; infectious disease (n=4/8; 50%) among gorillas and; infectious disease and disorders of the respiratory system (n=3/8; 38% each) among orangutans. Disease of the circulatory system (also referred to as cardiovascular disease) was the main cause of death among: adult (n=9/14; 64%) and aged bonobos (n=4/6;

67%); adult (n=15/50; 30%) and aged (n=15/46; 33%) chimpanzees and; among aged (n=8/30; 27%) gorillas. The main cause of death among adult gorillas, however, was disease of the digestive system (n=10/26; 38%). For orangutans, the main causes of death were respiratory disease (n=6/21; 29%) among adults and neoplastic disorders (n=5/17; 29%) among aged animals (Table 2.6).

Table 2.6 Data relating to cause of death for adult and aged animals in all four taxa, displayed as absolute number (n), percentage (%) and total
The most frequently identified cause(s) of death for each age category and taxa is shown in blue text

	BONOBO					CHIMPANZEE					GORILLA					ORANGUTAN				
	Adult		Aged		TOTAL	Adult		Aged		TOTAL	Adult		Aged		TOTAL	Adult		Aged		TOTAL
	n	%	*n*	%		*n*	%	*n*	%		*n*	%	*n*	%		*n*	%	*n*	%	
Behavioural/ management						2	*4%*			***2***										
Circulatory	9	*64%*	4	*67%*	***13***	15	*30%*	15	*33%*	***30***	7	*27%*	8	*27%*	***15***	2	*10%*	4	*24%*	***6***
Digestive						9	*18%*	2	*4%*	***12***	10	*38%*	7	*23%*	***17***	3	*14%*			***3***
Endocrine								1	*2%*	***1***	1	*4%*			***1***					
External causes	1	*7%*			***1***	8	*16%*	8	*17%*	***16***						5	*24%*	1	*6%*	***6***
Genitourinary			1	*17%*	***1***	5	*10%*	5	*11%*	***10***	3	*12%*	2	*7%*	***5***					
Infectious	2	*14%*			***2***	3	*6%*			***3***	2	*8%*	2	*7%*	***4***			1	*6%*	***1***
Miscellaneous/other								6	*13%*	***5***			1	*3%*	***1***			2	*12%*	***2***
Musculoskeletal						2	*4%*	2	*4%*	***4***			1	*3%*	***1***					
Neoplastic						2	*4%*	3	*7%*	***5***	1	*4%*	6	*20%*	***7***	2	*10%*	5	*29%*	***7***
Neurological						3	*6%*			***3***	1	*4%*	1	*3%*	***2***			1	*6%*	***1***
Reproductive																3	*14%*			***3***
Respiratory	2	*14%*	1	*17%*	***3***	1	*2%*	4	*9%*	***5***	1	*4%*	2	*7%*	***3***	6	*29%*	3	*18%*	***9***
TOTAL	*14*		*6*		***20***	*50*		*46*		***96***	*26*		*30*		***56***	*21*		*17*		***38***

2.3.5.3 Cause specific mortality

Disease of the circulatory system: Cardiovascular disease was identified as the most frequent cause of mortality in bonobos, among which it was responsible for 34% (n=13/38) overall mortality. It was also the second most frequent cause of death among chimpanzees (n=37/211; 18%) and gorillas (n=15/102; 15%). It was associated with 65%, 30% and 27% of adult/aged bonobo, chimpanzee and gorilla deaths respectively. In contrast, however, cardiovascular disease was the fourth most frequently identified cause of death for orangutans, among which it was responsible for only 10% (n=6/60) overall mortality and just 16% (n=6/38) of adult/aged animal mortality.

Death due to external causes: Death due to external causes was the leading cause of overall mortality among chimpanzees (n=61/211; 29%); the second most frequent cause of death among gorillas (n=15/102; 15%) and orangutans (n=12/60; 20%) and; the third most frequent cause of overall mortality in bonobos (n=6/38; 16%). Animals in the combined perinatal, infant and juvenile age categories accounted for 67% of all deaths due to external causes.

Diseases of the respiratory system: Respiratory disease was the cause of death for 70 of the great apes under study. It was the leading cause of death among orangutans (n=16/60; 27%) and the second most common cause of death for bonobos (n=12/38; 32%). It was the third most common cause of death category among chimpanzees (n=32/211; 15%) and (jointly with perinatal disorders) gorillas (n=10/102; 10%). Respiratory disease was particularly prominent as a cause of death mostly in younger animals, being responsible for a quarter (n=39/152; 25%) of all (combined) perinatal, infant and juvenile deaths, and having a median age at death of 5.95 years. Nonetheless, deaths were reported across the age categories. Respiratory disease was the main cause of death among adult orangutans (n=3/8; 38%) and second most frequent cause of death among adult/aged bonobos (n=3/19; 16%).

Diseases of the digestive system: Digestive system disorders were the main cause of overall mortality among gorillas, being responsible for 23% (n=23/102) of all deaths. By comparison, deaths in this category for only 7% (n=14/211) and 8% (n=5/60) of chimpanzee and orangutan deaths respectively, and none of the bonobo deaths under study. They were, however, the second most frequent cause of mortality among adult chimpanzees (n=9/50; 18%).

Neoplastic disorders: Neoplasms/cancers were the third most frequent cause of mortality among orangutans, accounting for 12% (n=7/60) overall mortality and 18% of adult/aged animal mortality. By contrast neoplastic disorders were associated with only 2, 3 and 8% of overall mortality in chimpanzees, bonobos and gorillas respectively. More than 90% (n=19/21) of the animals in this cause of death category were adult or aged. Thirty eight percent (n=8/21) affected the female reproductive tract.

Data relating to each of these four cause of death categories, for all four taxa combined, are displayed in Table 2.7.

Table 2.7 Further information relating to age at death and the individual diseases and conditions in animals dying as the result of diseases of the digestive system, respiratory system, neoplastic disease and due to external causes (d: days; y: years)

Cause of death category	Age at death	Diseases and conditions identified *(n)*
Diseases of the digestive system *(n=42)*	25.24 ± 14.48y	Peritonitis due to gastrointestinal inflammation (bacterial, protozoal) +/- perforation (13)
		Gastrointestinal inflammation (enteritis, colitis) (18)
		Hepatic disease (2)
		Pancreatitis (2)
		Gastrointestinal bloat (1)
		Dental disease (1)
		Necrotising oesophagitis (1)
		Intestinal invagination (1)
		Gastric ulceration (1)
		Gastroesophageal reflux (1)
		Appendicitis (1)
External causes *(n=94)*	1.15y (0d; 50.15y)	Trauma inflicted by conspecifics (63)
		Accidental deaths including drowning (16); entanglement in enclosure furniture (2); falling from a height (2)
		Anaesthetic deaths (6)
		Starvation due to delayed maternal rejection/inadequate milk supply (2)
		Escape associated (1)
		Self-induced injury (1)
		Allergic (anaphylactic) reaction (1)
Diseases of the respiratory system *(n=70)*	5.95y (2d; 61.78y)	Infection related bronchopneumonia/pneumonia (often bacterial +/- viral) (54)
		Upper respiratory tract infections, e.g. sinusitis, air sacculitis (6)
		Airway obstruction (2)
		Aspiration pneumonia (2)
		Airway disease incl. bronchitis, chronic obstructive pulmonary disorder (2)
		Throat abscess (1)
		Pleuritis, pleurisy (1)
		Chest deformation associated with rickets (1)
		Unknown (1)
Neoplastic disease *(n=21)*	34.21y (3.96; 50.21y)	Uterine/ovarian neoplasia (8)
		Mammary neoplasia (2)
		Hepatic neoplasia (2)
		Various organs (no histological diagnosis) (2)
		Sarcoma of the neck (1)
		Myeloid leukaemia (1)
		Multiple myeloma (1)
		Disseminated T cell lymphoma (1)
		Pelvic/perineal/inguinal fibrolipoma (1)
		Mandibular squamous cell carcinoma (1)
		Renal neoplasia (1)

2.4 Discussion

Zoo-housed great apes play an important role in conservation. Having a sound understanding of the diseases to which they may succumb is imperative to maintaining their optimal health and welfare. However, large-scale multi-centre and up-to-date published reviews of captive great ape mortality are lacking, particularly for bonobos and European zoo-housed chimpanzees, gorillas and orangutans (Strong et al. 2016). The aim of this study was therefore to carry out a robust, widespread, and up to date review to identify the main patterns of mortality in each of these populations.

Age at death analysis revealed that many great apes housed in zoos are far outliving the life expectancy of their wild counterparts (Bronikowski et al. 2011; Furuichi et al. 1998). Almost one quarter (23%) of the animals under study were aged/elderly at the time of death; a figure which can be expected to rise with ongoing improvements in captive husbandry. The management of an ageing great ape population can be expected to present new challenges, not least an increase in the incidence of chronic and age related diseases. This might be one explanation for why cardiovascular disorders in particular were found not only in the study population, but also among North American zoo-housed chimpanzees and gorillas (Gamble et al. 2004; Meehan & Lowenstine 1994). Interestingly, a survey of mortality among the SSP population also identified cardiovascular disease to be a prominent cause of mortality among orangutans (Lowenstine et al. 2008); a conclusion that was not drawn by this study.

Despite some animals' prolonged longevity, early years' mortality still accounted for a significant proportion of the deaths under study, with a quarter of all deaths occurring in animals less than twelve months of age. The perinatal period was identified as a period of high risk for death, and the causes identified as birth complications, maternal factors and trauma. This suggests, therefore, that any efforts to reduce these early deaths would need to focus not

around disease control, but instead around factors associated with pregnancy/birth management and perinatal husbandry practices. In instances of maternal rejection, poor milk supply or infant illness, whereby intervention would require separation of the dam and infant, however, the decision regarding whether or not to intervene must be balanced against other factors including: the potential long term behavioural consequences on the infant (such as imprinting on humans); the time and labour commitments associated; the longer term prospects and breeding potential of the animal itself and; the negative impact that separation could have on the dam not only at the time but also on her likelihood of breeding again in the future. From a conservation point of view, the decision should also be made following discussion with the species coordinators, taking into account the status of the current captive population and the impact that such a loss might have on its future growth and sustainability.

Infant deaths occurring outside of the perinatal period were most often caused by trauma (by dam or other conspecifics) or respiratory disease. Similar findings were also reported by studies of mortality among North American zoo-housed gorillas, chimpanzees and orangutans (Gamble et al. 2004; Lowenstine et al. 2008; Meehan & Lowenstine 1994). Unlike in the latter of these studies, however, parasitic infection (strongyloidiasis) was not identified as a cause of death in any infant or juvenile orangutans. Infanticide is a recognised issue in great apes both in captivity and in the wild (van Coillie et al. 2008; Courtenay 1988; Williams et al. 2008). Although research has been conducted, the potential reasons and risk factors behind it are not yet fully understood. In many cases, it might be unavoidable to some extent in group housed situations. However, if found to be impacting upon population growth and sustainability, a review of husbandry practices might be warranted.

Respiratory disease, especially in bonobos and orangutans, was associated with significant mortality especially among infants but also across the age categories. The high proportion of

deaths occurring due to respiratory disease in orangutans can be attributed to their susceptibility to air sacculitis, the occurrence, risk factors and pathogenesis of which are well documented in the literature (Cambre et al. 1995; Lawson et al. 2006; Zimmermann et al. 2011). The reasons behind the high prevalence of respiratory disease among bonobos, however, is less clear, and further research is warranted. Of particular interest is the role that infectious agents as primary and/or secondary pathogens, as well as environmental conditions and genetic factors might play. Further study into these topics would require dedicated prospective collection of relevant data and samples. In the interim, however, it may be possible to issue basic and presumptive recommendations for the prevention, investigation and/or treatment of morbidity and mortality caused by respiratory disease based on the risk factors known in other species (section 2.4.2.1).

Unique to gorillas, was the significant proportion of deaths associated with disorders of the digestive system, a finding also reported in the North American zoo-housed gorilla population (Meehan & Lowenstine 1994). Whilst infectious pathogens are often identified at post-mortem examination of these animals, care must be taken not to interpret their mere presence as being causal in every case. Gorillas are very susceptible to stress and when subjected to it, the levels of for example *Balantidium coli* will multiply. Although the consequence is inflammation and diarrhoea, it might be the stress (for example change in social situation, fighting) that was the inciting cause and therefore should be the focus for future prevention. The aetiology and pathogenesis of gorilla digestive disorders is however very complex, and is therefore an interesting area for further research.

2.4.1 Limitations of the study

2.4.1.1 Data collection

The retrospective and observational nature of this study meant that raw data recording was not designed specifically for purpose. The authors therefore had no control over the methods of data collection, recording system nor terminology used. To a certain extent, this has the potential to affect the reliability of the findings but is an issue that inherent to all studies of this type and is largely unavoidable (Senior, 2013). To minimise any potential impact that this might have upon the results, complete raw datasets (full post-mortem reports) as opposed to mere statements relating to the animals' cause of death were obtained where possible and reviewed in detail. Further qualitative information was also requested when required to assist with clarification. Nonetheless, there was great variation between zoological institutions with regards the approach to post-mortem investigation, specifically: the frequency/consistency with which macroscopic post-mortem +/- histopathological examination was performed; the organs examined; the samples collected/examined; any additional diagnostic tests carried out; the level of expertise of the individual performing the examination; the quality and detail of the written report; and the vocabulary and terminology used in the pathological descriptions and diagnoses. Greater consistency in these factors was identified in bonobo post-mortem reports than any other, however, this is likely to be related to the relatively small number of collections across which they are housed.

The main issues with these inconsistencies are two-fold: Firstly, valuable information is often missed and secondly, it can limit the power of conclusions drawn by large scale multi-centre studies such as this one. Standardisation of post-mortem data collection across the EEPs/SSPs would not only facilitate future studies of this type but also allow for ongoing monitoring of mortality over time and comparison within and between these and other populations.

Participation rates were excellent for a study of this size. Nonetheless, there were some gaps in the data owing to a lack of response by some zoological collections or in some instances, due to records being missing or unobtainable, for example due to a change in staffing. Prospectively, the collection of post-mortem reports ideally at the time of death or in a periodic manner (for example annually) would reduce the amount of missing data in such studies and allow for any gaps or inconsistencies to be identified and rectified at the time.

It was found to be common in the event of foetal or perinatal losses for the dam to carry her offspring for some time before relinquishing the body meaning that a significant degree of autolysis had already taken place before the carcass was acquired or presented for post-mortem examination. Post-mortem carcass damage by other animals was also a relatively common occurrence. Both these factors were quoted as reasons for a lack of post-mortem data availability for several animals in this study, meaning that valuable information relating to these deaths was lost. Whilst in many instances, this loss of information is unavoidable, carrying out a basic examination could still result in meaningful information being gathered. For example, it could allow true foetal losses to be distinguished from early perinatal deaths; premature births to be distinguished from those carried to full term and; dams which experienced dystocia to be identified and therefore managed/monitored accordingly for future births.

Even in the absence of significant carcass autolysis or damage, there were some animals for which a post-mortem examination was not carried out because either the cause of death was already known or because the animal was elderly. However, the purpose of the post-mortem examination is not merely to identify the cause of death of an animal. It allows the detection of concurrent and contributing conditions of significance; monitoring of disease prevalence and; the identification of emerging diseases.

2.4.1.2 Data processing

Cause of death category definitions were closely adhered to. Utilisation of the online ICD-10 system facilitated this and limited the potential for subjectivity. The ICD-10 is the tenth revision of the International Statistical Classification of Diseases and Related Health problems; a medical classification list which has been developed by the World Health Organisation for use in categorising diseases, lesions, signs and symptoms. Use of the online ICD-10 browser function allowed for certain diseases to be searched for, and for any uncertainties to be clarified allowing appropriate categorisation of deaths according to their cause. Nonetheless, a certain degree of ambiguity remained for some individuals, especially those which were found to have multiple equally severe or extensive coexisting conditions on post-mortem examination. This was particularly true, for example, of elderly animals which had been euthanased due to a generalised deterioration in health and quality of life.

Categorisation for the purposes of quantitative analysis can also result in loss of qualitative detail. This is particularly true for those deaths for which the cause of death is ill-defined, for example in cases of perinatal or peri-anaesthetic death. Another way in which categorisation can result in a loss of qualitative detail is demonstrated by the death of an 11-year old male bonobo: Neutrophilic meningitis was diagnosed on post-mortem examination, but since it had occurred secondarily to a severe streptococcal respiratory infection, his death was classified as being respiratory in origin. However, this animal was also diagnosed with a concurrent cardiomyopathy, which might have for example have affected his ability to combat and overcome an otherwise mild illness and ultimately therefore have contributed to his death. This case therefore demonstrates not only a loss of qualitative detail, but also highlights the importance of a full necropsy even when the cause of death is known, to identify coexisting and/or contributing conditions.

2.4.2 Implications of findings

In this study, data from a total of 681 deaths were included, making it the largest study of its type to date. In addition, the multi-centre nature of this study and the excellent participation rate achieved means that the data collected can be considered representative of the wider population. The recent time-period studied also deems the findings up to date and relevant.

The implications of this study are many, stretching across the day-to-day management and veterinary care of great apes housed in zoos; prospective data collection and processing; future research into zoo-housed great ape health and disease and; wild great ape conservation.

2.4.2.1 Daily management and veterinary care of zoo-housed great apes

On the individual zoological collection level, the findings of this study can be used to inform future management practice and policy and ultimately therefore enhance the health and welfare of zoo-housed great apes. When considering perinatal mortality, for instance, there suggests a need for zoos to ensure the following takes place: careful planning of pregnancy and management of breeding; early identification of pregnancy and close monitoring of pregnant animals to maximise maternal health; careful management of peri-parturient animals to minimise stress; prior planning for potential complications such as dystocia; provision of a suitable environment for birth and/or nursing and careful timing of separation from/mixing with conspecifics. Especially for chimpanzees, the findings also imply a need for careful management of social situations and especially the introduction of unfamiliar cage-mates, to limit the potential for fight/attack related deaths. For gorillas, there is also a great need for careful management of those individuals deemed to be at risk of developing gastrointestinal upsets, for example: those which are under stress, being moved, or undergoing anaesthesia.

The findings of this study will also be informative to the practicing clinician, equipping them with a sound understanding of the diseases with which they might be presented. It highlights a selection of diseases or clinical presentations which require immediate and, in some cases, intensive management, including for example: respiratory symptoms in one/more bonobos or a gorilla with gastrointestinal symptoms (for example: diarrhoea or anorexia). It also suggests to them key areas to be incorporated into their routine preventative healthcare plans. For example:

1. Routine screening of all adult bonobos, chimpanzees, gorillas +/- orangutans for cardiac disease
2. Regular screening of gorillas for gastrointestinal parasites and monitoring of faecal consistency
3. Abdominal ultrasound for the identification of reproductive or gastrointestinal neoplasms, especially in elderly orangutans
4. Assessment and, where indicated, treatment of elderly animals for age related and chronic diseases
5. Close monitoring of traumatic wounds and treatment of complications where necessary, especially in chimpanzees
6. Prevention and management of respiratory disease, especially bonobos. For example:
    - Limiting/preventing the potential for contact with respiratory secretions with personnel (for example, keepers wearing masks)
    - Vaccination of staff and/or animals where possible (for example against seasonal influenza)
    - Early treatment of respiratory symptoms with antimicrobial agents where appropriate and symptomatic relief to limit mortality associated with secondary bacterial infections

A pro-active approach to the above would greatly be facilitated by animals being trained, for example, for: conscious blood pressure, ultrasound and echocardiographic assessment; body part presentation; wound flushing etc. Where possible therefore, training great apes for these behaviours through operant conditioning should be included in any husbandry/preventative healthcare plan.

2.4.2.2 Further research

The identification of the main causes of death among each of the four great ape taxa has also highlighted several key areas requiring additional research to further understanding about the aetiology, epidemiology and pathogenesis of:

- Cardiovascular disease, especially among adult chimpanzees, bonobos and gorillas
- Respiratory disease, especially among bonobos
- Digestive system disorders in gorillas

The raw post-mortem data collected as part of this study has been compiled in an electronic database. There is scope for further analysis to be carried out on this data as a first step to glean additional information about the epidemiology of the diseases of greatest significance. However, it is also recommended that the proactive collection of prospective data and biological samples of future potential use and interest is initiated, ideally by the relevant EEPs.

2.4.2.3 Monitoring of mortality; data collection and processing

The methodology used in this study is both comprehensive and repeatable, enabling it to serve as a model for future studies of its type in these and other species. The findings of the study will lay the foundations for the ongoing monitoring of mortality among zoo-housed great apes in the future, through which new and emerging disease patterns can be identified.

Prospectively, zoos should be encouraged to perform as full and comprehensive post-mortem examination as possible, in the event of every great ape death, regardless of the animal's age or whether or not the cause of death is already known. It is also suggested that post-mortem reports are collected ideally at the time of death or in a periodic manner (for example annually) to reduce the amount of missing data in such studies and allow for any gaps or inconsistencies to be identified and rectified at the time. A named and dedicated individual should be responsible for this task, which should be directed and facilitated by the species coordinators and veterinary advisors. The timely collection along with the centralised recording of post-mortem data would also help to promote the consistency in categorisation of the cause of death and comparison of findings over time: Deaths should be coded at the time and inputted to a database to limit the potential for bias to be introduced at a later date. It is recommended that the same classification system used for this review, which was adapted from the ICD-10 classification system, is utilised for this purpose for ease, accuracy and consistency.

The reliability of the findings of such studies would be greatly enhanced if the approach to post-mortem examination and subsequent data collection and recording was standardised across collections. It is suggested, therefore, that a recommended process for the post-mortem examination of great apes including a list of organs to be examined and information to be gathered is generated and in incorporated into the best practice guidelines for each species. It is also recommended that a minimum dataset of information to be gathered in the event of

foetal and perinatal deaths is also generated and shared in a similar fashion. The existence of these protocols and the importance of their implementation should be brought to the attention of key individuals (vets, pathologists, curators) working in great ape holding zoological collections and support given where needed (for example by smaller zoos with no on-site veterinary staff).

2.4.2.4 Conservation of wild populations

The opportunity to study wild great ape mortality is limited: sightings can be infrequent; the animals infrequently show overt clinical signs of disease; and fresh biological samples are scarce. Infectious and respiratory diseases in particular have been identified as a major concern for the future sustainability of wild great ape populations (IUCN 2012; Sakamaki et al. 2009; de Wachter et al. 2003; Walsh et al. 2003). As human-animal contact continues to increase through tourism, hunting, research and conservation activities, and because of the growing human population, this threat can be expected to rise. Information gleaned from studying zoo-housed great apes can therefore be very valuable in providing an insight into the health, and therefore the conservation, of their wild counterparts.

CHAPTER 3:

A RETROSPECTIVE REVIEW OF GREAT APE CARDIOVASCULAR DISEASE EPIDEMIOLOGY AND PATHOLOGY

3.1 Introduction

Great ape cardiovascular disease is a common topic for discussion within the zoological literature (Chapter 1), and has been identified as a major cause of mortality among the European (Chapter 2) and North American (Gamble et al. 2004; Lowenstine et al. 2008; Meehan & Lowenstine 1994) zoo-housed great ape populations. Despite this, knowledge and understanding about its diagnosis, treatment and ultimate prevention, remain poor. This in turn, is fundamentally due to a lack of understanding about its epidemiology and pathogenesis. The need for further understanding in these areas in particular, formed the basis for this chapter of the thesis.

This retrospective study explores the frequency and patterns of cardiovascular disease associated mortality in zoo-housed great apes. It aims to identify potential risk factors that might be involved in the aetiopathogenesis of great ape cardiovascular disease.

3.2 Materials and methods

3.2.1 Study population

All (n=71) those bonobos, chimpanzees, western lowland gorillas and orangutans that had been identified as dying due to cardiovascular disease in a prior retrospective review of mortality between 1st January 2004 and 31st December 2014 (Chapter 2) were included in this study.

3.2.2 Data collection and processing

For each animal, the following information was entered to an electronic spreadsheet: animal identification information (regional studbook number; name), species; zoological collection at the time of death; sex and; age at death (exact in years; age category; 5-year age interval). Estimated ages and age categories already defined in Chapter 2 were again used.

The post-mortem reports for each of these animals were reviewed in detail and key information relating to the manner of death and cardiovascular disease responsible were extracted. The manner (nature and circumstance) of death was classified as: euthanasia; peri-anaesthetic; natural (sudden) or; natural (following period of illness). Peri-anaesthetic deaths were those that occurred following anaesthetic induction, intra-operatively or during anaesthetic recovery, or that occurred after the animal not regaining full consciousness post-anaesthesia. Deaths were classified as sudden if they were non-violent (i.e. non-traumatic), unexpected and occurred within 24 hours of the animal last being seen in a stable, apparently healthy condition (Virmani et al. 2001). The main cardiovascular lesion associated with death was categorised according to the criteria described in Table 3.1.

Table 3.1 Categories of cardiovascular disease, their codes, equivalent International Classification of Diseases (ICD-10) category, and examples

Category	**Code**	**Examples**	**Equivalent ICD-10 category**
Hypertensive diseases	HTD	Primary hypertension, hypertensive heart disease, secondary hypertension	I10-I15
Ischemic heart diseases	IHD	Myocardial infarction, chronic ischemic heart disease, atherosclerotic heart disease, aneurysm, ischemic cardiomyopathy	I20-25
Cerebrovascular diseases	CVD	Cerebral infarction; subarachnoid haemorrhage; intracerebral haemorrhage other non-traumatic intracranial haemorrhage; cerebral infarction	I60-I69
Diseases of ***arteries, arterioles and capillaries***	ART	Atherosclerosis; aneurysm and dissection; arterial embolism and thrombosis;	I70-I79
Diseases ***of veins, lymphatic vessels and lymph nodes****, not elsewhere classified*	VEN	Phlebitis and thrombophlebitis; venous embolism and thrombosis	I80-I89
Pulmonary heart disease *and diseases of* ***pulmonary circulation***	PUH	Pulmonary embolism; pulmonary hypertension; pulmonary artery aneurysm	I26-I28
Pericardial disease	PER	Pericarditis; haemopericardium not classified elsewhere	I30-I32
Endocarditis	END	Endocarditis; infectious or non-infectious	I33, I38-I39
Valve diseases	VAL	Non-inflammatory disorders (i.e. not endocarditis) of the aortic, pulmonary, tricuspid or aortic valve incl. dysplasia; insufficiency; stenosis	I34-I37
Myocarditis	MYC	Acute; chronic; infectious; non-infectious	I40-I41
Cardiomyopathy	CDM	Hypertrophic cardiomyopathy; dilated cardiomyopathy; nutritional cardiomyopathy; restrictive cardiomyopathy; endomyocardial (eosinophilic) disease	I42-I43
Conduction disorders	CDD	Atrioventricular and bundle branch blocks; heart blocks	I44-I45
Cardiac arrhythmias	CAR	Atrial fibrillation/flutter; paraxoysmal tachycardia; ventricular fibrillation	I47-I49
Heart failure	HFF	Congestive heart failure; left heart failure	I50

Cardiomyopathies were further sub-categorised by diagnosis, as stated in the post-mortem report. Sub-categories used were as follows: dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM); and arrhythmogenic right ventricular cardiomyopathy (ARVC) (Davies 2000; National Heart Lung and Blood Institute 2016; Richardson et al. 1996). Those cardiomyopathies which did not conform to any of these diagnoses were categorised as 'unclassified'. The pathological descriptions stated in the post-mortem reports of those animals dying due to an unclassified cardiomyopathy were subject to thematic analysis: All text contained within the macroscopic and histopathologic descriptions relating to the heart was reviewed and initial codes generated. Descriptive themes were identified and defined, and organised into thematic networks for interpretation.

3.2.3 Data analysis

Statistical analyses were carried out using Microsoft Excel (Microsoft 2010) and GraphPad Prism 7 for MacOS X (2016). Data were assessed for normality using the Shapiro-Wilk test. Normally distributed data are expressed as mean and standard deviation, and non-normally distributed data as median (min; max) unless stated otherwise. One-way ANOVA was used to assess for differences in age at death between taxa. A Fisher's exact test of independence was carried out to assess for sex variation in risk of death. Confidence intervals were set at 95% and p-values of <0.05 were considered significant.

3.3 Results

3.3.1 Age at death

Age at death, due to cardiovascular disease, was found to be normally distributed across the taxa. It ranged from 7.80 to 52.57 years, with the youngest animal being a chimpanzee and the eldest, a bonobo (Table 3.2). One-way ANOVA revealed no statistically significant difference in age at death due to cardiovascular disease between the taxa. More than 90% (n=64/71) of animals were adult or aged at the time of death, with three quarters (n=53/71; 75%) being aged >25 years.

3.3.2 Manner of death

The death of 55 animals were categorised according to the nature and circumstances (Table 3.3). Records were not adequate to determine manner of death for the remaining 16 animals. Twenty percent (n=11/55) of the deaths were due to euthanasia and 29% (n=16) were peri-anaesthetic in nature. Five (9%) of the deaths followed a period of illness, ranging in duration from 24 hours to 6.5 years. The biggest proportion (n=23/55; 42%) of deaths, however, were sudden or unexpected; that is that the animals were found dead with no signs of cardiac ill health being observed in the preceding 24 hours.

Although not analysed as part of this study, more in depth review of the medical records for animals which had died suddenly was also carried out, in instances where such information was available. Contained within some of these historical records were events that might, with hindsight, now be interpreted as being an indicator that the animal was suffering from cardiac disease. These include, for example: unexplained syncopal episodes or ‘vacant’ moments; prolonged anaesthetic recoveries and; a change in social status or perhaps even increase in aggression from conspecifics.

Table 3.2 Age at death analysis (including age category and 5-year age interval) of animals (all four taxa) dying due to cardiovascular disease

		Bonobo	Chimpanzee	Gorilla	Orangutan	ALL
Number of animals		13	37	15	6	71
Age at death	***Range** (min; max)*	21.56; 52.57	7.80; 51.35	17.93; 44.71	22.64; 50.78	7.80; 52.57
	Mean ± SD	32.25 ± 7.94	30.62 ± 11.26	33.66 ± 7.72	41.76 ± 11.67	32.50 ± 10.35
Age category at death	***Juvenile***	0	1	0	0	1
	Sub-adult/ adolescent	0	6	0	0	6
	Adult	9	15	7	2	33
	Aged	4	15	8	4	31
Age interval at death	***5-10 years***	0	1	0	0	1
	10-15 years	0	1	0	0	1
	15-20 years	0	5	1	0	6
	20-25 years	2	6	1	1	10
	25-30 years	4	6	2	0	12
	30-35 years	3	3	3	1	10
	35-40 years	3	7	5	0	15
	40-45 years	0	4	3	1	8
	45-50 years	0	2	0	0	2
	50-55 years	1	2	0	3	6

Table 3.3 Data relating to the manner of death for great apes from all four taxa (n=55) dying due to cardiovascular disease

	Bonobo	Chimpanzee	Gorilla	Orangutan	*ALL*
Euthanasia *(n/total; %)*	3/11; 27%	6/27; 22%	1/13; <8%	1/4; 25%	***11/55; 20%***
Peri-anaesthetic *(n/total; %)*	1/11; 9%	10/27; 37%	3/13; 23%	2/4; 50%	***16/55; 29%***
Natural: sudden *(n/total; %)*	6/11; 55%	8/27; 30%	8/13; 62%	1/4; 25%	***23/55; 42%***
Natural: illness *(n/total; %)*	1/11; 9%	3/27; 11%	1/13; <8%	-	***5/55; 9%***

3.3.3 Cardiovascular diseases

The category of cardiovascular disease most frequently associated with death (n=38/71; 54%) were the cardiomyopathies (Table 3.4). Further data relating to these deaths are presented in Section 3.3.3.1. Also associated with a significant proportion of all cardiovascular mortalities were cerebrovascular disorders (n=11/71; 15%; cerebral haemorrhages and infarctions) and diseases of the arterial and capillary system (n=8/71; 11%). The latter of these, arterial disorders, were particularly common among gorillas (n=3/15; 20%; 2nd most common disease). They were also the cause of 15% (n=2/13) bonobo and 8% (n=3/37) chimpanzee, but not any orangutan cardiovascular mortalities. Three quarters (n=6/8; 75%) of the deaths which were categorised as being due to disease of the arterial and capillary system were aortic dissections or aneurysms, the remaining deaths (n=2) being due to arterial thrombosis.

Table 3.4 Data relating to the cardiovascular diseases associated with death for animals (n=71) across all four taxa, sub-categorised by cause

	Bonobo	**Chimpanzee**	**Gorilla**	**Orangutan**	**ALL**
Diseases of ***arteries, arterioles and capillaries***	2/13; 15%	3/37; 8%	3/15; 20%	-	**8/71; 11%**
Cardiomyopathy	7/13; 54%	23/37; 62%	7/15; 47%	1/6; 17%	**38/71; 54%**
Cerebrovascular disease	3/13; 23%	5/37; 14%	1/15; 7%	2/6; 33%	**11/71; 15%**
Ischemic heart disease	-	1/37; 3%	1/15; 7%	2/6; 33%	**4/71; 6%**
Pulmonary heart disease *and diseases of* ***pulmonary circulation***	-	1/37; 3%	-	-	**1/71; <1%**
Heart failure	1/13; 8%	1/37; 3%	2/15; 13%	1/6; 17%	**5/71; 7%**
Valve diseases	-	1/37; 3%	1/15; 7%	-	**2/71; <1%**
Endocarditis	-	1/37; 3%	-	-	**1/71; <1%**
Myocarditis	-	1/37; 3%	-	-	**1/71; <1%**

The four deaths which occurred due to ischemic heart disease were due to acute and/or chronic myocardial infarction. Two of these animals were also reported to have had significant atherosclerosis.

Five deaths due to 'cardiac failure' were reported. All had significant pathological lesions elsewhere in the body (renal, hepatic or respiratory) which might have been the inciting cause of the circulatory failure which ultimately resulted in death.

3.3.3.1 Cardiomyopathies

Cardiomyopathies were responsible for over half (n=38/71; 57%) of all cardiovascular deaths. They were responsible for 62, 54 and 47% of all deaths occurring due to cardiovascular disease in chimpanzees (n=23/37), bonobos (n=7/13) and gorillas (n=7/15) respectively. Cardiomyopathies were, however, identified as the cause of death for only 17% (n=1/6) of all orangutan cardiovascular disease deaths.

Manner of death: Information relating to the manner of death was available for >80% (n=31/38) of those deaths which occurred due to a cardiomyopathy. Euthanasia accounted for less than 10% (n=3/31) of these. Peri-anaesthetic mortality accounted for a further 29% (n=9/31); six (67%) of which occurred in the intra-operative period, two (22%) during anaesthetic recovery, and one (11%) 48 hours post-procedure, during which time the animal never fully regained consciousness. Only two (n=2/31; 6%) of the animals showed overt clinical signs of disease in the period leading up to their death: an elderly male bonobo had a 6.5year history of cardiac disease, which was first detected following change in a group behaviour (he was attacked by the females). On cardiac assessment, a reduction in ejection fraction, left ventricular hypertrophy and the presence of arrhythmias had been diagnosed, and

he had subsequently been receiving angiotensin converting enzyme (ACE) inhibitors, beta blockers and aspirin. He had, however, been clinically well and had shown no clinical evidence of deterioration in the period preceding his death. The second case was an elderly male gorilla, which had a 3-month history of progressive lethargy and respiratory signs (coughing, dyspnoea) presumed due to heart failure, for which he was receiving diuretic therapy. Unlike the bonobo, he had shown a deterioration in his health over the preceding two weeks but died before scheduled euthanasia could be carried out. Over half (n=16/31; 52%) of the deaths, however, had been sudden or unexpected, with the animals being reported as seemingly healthy in the 24 hours preceding death. The nature and circumstances surrounding of death of the remaining animal was unknown.

Diagnosis: Two cases of hypertrophic cardiomyopathy (HCM) were reported: one anaesthetic death in an adult male chimpanzee and one sudden death in an adult male gorilla. Two cases of dilated cardiomyopathy (DCM) were also reported. The first was in an elderly male chimpanzee which died during an anaesthetic procedure carried out to investigate recent weight loss and 'rapid ageing'. The second was in a young adult (16-year-old) male chimpanzee which also died whilst under anaesthesia to investigate prior collapsing episodes. One case of arrhythmogenic right ventricular cardiomyopathy (ARVC) was reported in a 17-year-old chimpanzee which died suddenly, and a diagnosis of restrictive cardiomyopathy (RCM) was made in an adult female bonobo which also died unexpectedly.

The remaining deaths (n=30/36; 83%) were categorised as occurring due to an unclassified cardiomyopathy. Often referred to as fibrosing cardiomyopathy within the post-mortem reports, these cardiomyopathies had one consistent feature; all were typified by the histological finding of an increase in fibrous connective tissue, However, as shown by thematic analysis (Appendix 8), the type and distribution of the fibrosis varied (for example: interstitial,

replacement, perivascular, dissecting and/or coalescing). In some, but not all, cases myocardial necrosis and/or active myocardial degeneration was reported. Myofibre changes described also varied, and included both thickened or enlarged myofibres as well as thin and wavy fibres. Cardiac myocyte nuclei were reported to be uniform in appearance, or to vary in size (anisocytosis) and shape (elongation) and in some cases exhibit atypia. In some, but not all, instances, the fibrotic lesions were also reported to be accompanied by cellular infiltration or inflammation, with a mixture of cell types being observed. Some of the hearts which were diagnosed with FCM were reported to appear normal on macroscopic examination, whilst others were mottled and pale pink or whitish in colour. In some instances, hearts were described as being 'enlarged' although it was often not always specified as to whether this enlargement was due to hypertrophy (physiological or pathological) or dilation. Such descriptions of an increase in heart size were infrequently accompanied by data relating to dimensions or weights, nor reported alongside the animal's bodyweight. Ventricular walls were reported in some animals to be hypertrophied and firm/tough, whereas in others, thinned and dilated. Where hypertrophy was diagnosed, it was rarely specified whether this was concentric or eccentric in type. The changes were reported to affect various regions of the heart: the left and right atria, left and right ventricles and interventricular septum. However, details relating to the specific myocardial distribution (sub-endocardial; mid-myocardial; sub-epicardial; trans-myocardial) of the lesions were rarely provided. The chronicity of the lesions reported ranged from acute to chronic. The extent and severity of the changes varied from being mild and focal to severe and extensive, affecting significant portions of the myocardium.

3.3.4 Sex variation

3.3.4.1 Sex variation in proportional mortality: adult/aged animals

Cardiovascular disease was associated with a greater proportional mortality among adult and aged males than females across all four taxa (Table 3.5). For all four taxa combined, the relative risk of death due to cardiovascular disease associated with the male sex was 2.4. This discrepancy was most obvious for gorillas, among which males were >8 times more likely to die as the result of cardiovascular disease than females. For chimpanzees, the risk for males was almost twice that of females. Although still evident in bonobos and orangutans, the sex variation in risk of cardiovascular death was not statistically significant.

Table 3.5 Data relating to sex variation in risk of death due to cardiovascular disease between male (M) and female (F) great apes from all four taxa

	Bonobo		**Chimpanzee**		**Gorilla**		**Orangutan**		**ALL**	
	M	***F***	***M***	***F***	***M***	***F***	***M***	***F***	***M***	***F***
Deaths due to cardiovascular disease: *n/total; %*	9/12; 75%	4/8; 50%	17/39; 44%	13/57; 23%	13/25; 52%	2/31; 6%	3/17; 18%	3/21; 14%	42/93; 45%	22/112; 20%
Relative risk	1.50		1.91		8.06		1.24		2.40	
P value	0.3563		0.0435		0.0002		>0.999		<0.0001	
Statistical significance[1]	ns		*		***		ns		****	
95% confidence interval	0.76 to 3.63		1.06 to 3.46		2.33 to 30.25		0.31 to 4.82		1.56 to 3.73	

[1] *ns: p>0.05, not statistically significant; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001*

Table 3.6 Data relating to sex variation in age at death due to cardiovascular disease between male (M) and female (F) great apes from all four taxa

		Bonobo		Chimpanzee		Gorilla		Orangutan		ALL	
		M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Age at death *(mean ± SD)*		30.33 ± 5.28	36.59 ± 11.91	32.12 ± 10.31	28.85 ± 12.36	32.64 ± 7.74	40.30 ± 3.83	42.08 ± 9.00	37.43 ± 12.87	***32.58*** ± 8.85	***31.91*** ± 12.16
T-test	*P value*	0.3770		0.3935		0.1288		0.6379		***0.8077***	
	Summary[1]	ns		ns		ns		ns		***ns***	

[1] *ns: p>0.05, not statistically significant*

Table 3.7 Data relating to sex variation in cardiovascular condition associated with death for male (F) and female (F) great apes from all four taxa, displayed as absolute number (n) and percentage (%) of total

	Bonobo		Chimpanzee		Gorilla		Orangutan		ALL		
	M	***F***	***M***	***F***	***M***	***F***	***M***	***F***	***M***	***F***	***Fisher's exact test***[1]
Arterial/capillary	*2; 22%*	*0*	*1; 5%*	*2; 12%*	*3; 23%*	*0*	*0*	*0*	*6; 13%*	*2; 8%*	*RR M>F: 1.73 (ns)*
Cardiomyopathy	*5; 56%*	*2; 50%*	*18; 90%*	*5; 29%*	*6; 46%*	*1; 50%*	*0*	*1; >33%*	*29; 64%*	*9; 35%*	*RR M>F: 2.00 ** *
Cerebrovascular	*2; 22%*	*1; 25%*	*1; 5%*	*4; 24%*	*1; <8%*	*0*	*1; 33%*	*1; >33%*	*5; 11%*	*6; 23%*	*RR F>M: 2.08 (ns)*
Ischemic	*0*	*0*	*0*	*1; <6%*	*1; <8%*	*0*	*2; 67%*	*0*	*3; 7%*	*1; 4%*	*RR M>F: 1.73 (ns)*
Pulmonary	*0*	*1; 25%*	*0*	*1; <6%*	*1; <8%*	*1; 50%*	*0*	*1; >33%*	*1; 2%*	*4; 15%*	*RR F>M: 6.93 (ns)*
Other	*0*	*0*	*0*	*4; 24%*	*1; <8%*	*0*	*0*	*0*	*1; 2%*	*4; 15%*	-
TOTAL	***9***	***4***	***20***	***17***	***13***	***2***	***3***	***3***	***45***	***26***	***-***

[1] *ns: p>0.05, not statistically significant; ** p ≤ 0.01*

3.3.4.2 Sex variation in age at death

There was no statistically significant difference in age at death due to cardiovascular disease between male and female great apes (Table 3.6).

3.3.4.3 Sex variation in cardiovascular disease

Cardiomyopathies were responsible for 64% of all male compared with only 35% of female cardiovascular disease deaths (Table 3.7); the male gender being associated with a >2-fold increase in risk of death due to cardiomyopathy (p=0.0089; CI: 1.19 to 3.71). This sex difference was most notable for chimpanzees, among which 90% (n=18/20) of male cardiovascular disease deaths were due to a cardiomyopathy, compared with only 29% (n=5/17) of females (relative risk, M>F: 3.06; p=0.0002; CI: 1.63 to 6.85).

Arterial diseases were only reported among male, not female, bonobos and gorillas. They were however, associated with 12% of female cardiovascular disease deaths compared with only 5% of those in male chimpanzees.

3.4 Discussion

3.4.1 Age at death

Cardiovascular disease was found to predominantly be a disease of adult and aged animals, with the mean age at death for all taxa combined being 32.5 years (range: 7.8 to 52.6y). Given that advancing age is a well-known risk factor for heart disease in humans (Ho et al. 1993), this finding was not unexpected. Nonetheless, cardiovascular disease was also associated with the death of seven chimpanzees under 20 years of age. The future reproductive potential of an animal of this age means that the impact of its death on the growth and sustainability of the population could be great. The possibility that chimpanzees might be affected by heart disease at a younger age should therefore be monitored closely, and potential reasons for it explored. Given that the period of adolescence and sub-adulthood, especially for males, is associated with an increase in reproductive behaviour, intense sexual competition, and often also changes in social hierarchy, the potential interplay between sex, stress and disease risk in this sub-population, in particular, would be an interesting avenue for future research.

3.4.2 Male sex and cardiovascular disease risk

As is the case in other species, male sex was identified by this study as a risk factor for death due to cardiovascular disease in great apes. As reviewed by Pérez-López et al. (2010), various hormonal, biochemical and genetic factors might responsible for this sex variation in risk. Both oestrogen and testosterone have been shown to be cardioprotective (Bain 2007; Pérez-López et al. 2010; Rosano & Panina 1999; Tsang et al. 2007). Their reduction, therefore, whether naturally occurring as the result of ageing, due to social factors or contraception, might be associated with an increased risk of cardiovascular disease, therefore making this another important topic for further investigation.

The increased risk of death due to cardiovascular disease in males when compared with females might also be responsible for the female survival advantage observed among bonobos, chimpanzees and gorillas (Chapter 2). Males have been reported to experience higher mortality, more rapid ageing and reduced longevity across the primate species (Bronikowski et al. 2011; Hill et al. 2001; Reinartz et al. 2002; Schubert et al. 2013), with numerous physical, social and behavioural explanations for this being proposed. The most popular theory is that, in polygamous species, intense sexual competition requires males to invest in mating success and reproductive fitness at the cost of health maintenance and longevity (Clutton-Brock & Isvaran 2007; Rolff 2002). Sex variation in cardiovascular disease risk might also be stress related. In bonobo society, for example, males tend to occupy the lowest social ranking positions (Stevens et al. 2007; Surbeck & Hohmann 2013) and have also been found to have higher salivary cortisol and alpha amylase levels than the females (Behringer et al. 2013; Hohmann et al. 2009). It could be hypothesized therefore that male bonobos experience greater long term stress than do their female counterparts and that this impacts upon their health and ultimately their survival. Male bonobos (like adolescent/sub-adult chimpanzees), would therefore be another interesting sub-population in which the interplay between social status, sex and disease would be an interesting focus for further investigation.

3.4.3 Species variation in risk of death due to cardiovascular disease

The findings of this study suggest that orangutans are far less susceptible to death due to cardiovascular disease than the other three great ape taxa. This might explain why orangutans are the only taxa among which mean life expectancy for males was greater than that for females (Chapter 2). It is not clear whether the marginally higher overall life expectancy among orangutans when compared with the other great ape taxa (Chapter 2), is the cause or an effect

of their reduced susceptibility to cardiovascular disease, but it challenges any suggestion that great ape cardiovascular disease is merely be an inevitable feature of ageing.

Orangutans differ from the other great ape taxa in several ways (Caldecott & Miles 2005). Firstly, they are the only great apes to inhabit South East Asia, the other taxa all being endemic to Africa. The orangutan is also predominantly frugivorous, their diet consisting of a much higher proportion of fruits and seeds than the more folivorous and omnivorous gorilla, bonobo and chimpanzee. Unlike the other taxa, which exist usually in family groups, the orangutan is a largely solitary species. They are also an arboreal species, spending a much greater proportion of their time in the trees than on the ground than other greats. Finally, orangutans are also genetically very distinct from the African great apes and humans, which are grouped together in the *Homininae* subfamily. The role that these, and many other factors, might play in the orangutan's apparent reduced propensity to develop cardiovascular disease are interesting areas for future research.

3.4.4 Manner of death

Many of the great apes in this study did not display any clinical signs of ill health prior to their death, which was subsequently therefore categorised being sudden in nature. That is not to say, however, that they were truly free of clinical signs or symptoms. The animals might have been experiencing for example, feelings of light headedness or palpitations, which they were not able to communicate, or might even have intentionally masked. It was for this reason, that the definition of sudden death used by Virmani et al (2001), whereby the deceased was last seen in a stable condition less than 24 hours previously was deemed most appropriate for use in this study.

It is possible, that some very subtle signs of suboptimal cardiac health do exist (Section 3.3.2), an understanding of which requires more in depth study of clinical records assessing for any correlation between ante- and post-mortem findings. However, the question remains; what action a clinician might take if informed of a suspected heart condition in a great ape. Due to their size and dangerous nature, a full health assessment would require the animal to be anaesthetised, but this study identified cardiovascular disease as a risk factor for peri-anaesthetic death. The risk of death, is of course, not a reason to take no action at all, but suggests that the veterinarians, keeping and curatorial staff should be well informed of the potential consequences of carrying out such procedures on these animals. An appreciation for the risk of anaesthetic death must also be considered when anaesthetising any great ape that falls within the subpopulations identified by this study as being at particularly high risk, even in the absence of cardiac symptoms or other cause for concern. Clinicians must, at very least, be knowledgeable about the cardiovascular effects that the anaesthetic drugs they are using can have on the cardiovascular system (Chapter 5), and be equipped to deal with them should they occur.

3.4.5 Cardiomyopathies

This study concluded that the most common cardiovascular diseases to be associated with mortality were the cardiomyopathies. Cases of hypertrophic (HCM), dilated (DCM), restrictive (RCM) and arrhythmogenic right ventricular (ARVC) cardiomyopathies were identified. More commonly, however, great apes were reported to be affected by a condition (often called fibrosing cardiomyopathy, FCM), which does not fit into any of these well recognised categories or diagnoses (Davies 2000; National Heart Lung and Blood Institute 2016; Richardson et al. 1996).

The term FCM was first used in 1995 to describe myocardial replacement fibrosis with atrophy and hypertrophy of cardiac myocytes, absent to mild myocardial inflammation, with no apparent aetiology or associated disease condition in eleven captive male western lowland gorillas (Schulman et al. 1995). It has not since been further defined nor characterised in any greater detail. Its association with ante-mortem symptoms and other cardiovascular disorders, such as atherosclerosis and hypertension, have also been poorly studied. It is not a term that is recognised or used in any other clinical or pathological context, whether veterinary or medical. Despite this, and as shown by this study, its use as a pathological diagnosis has been widely accepted among the zoo profession. As shown by this study, the term is applied to many, distinct, sometimes conflicting, pathological lesions which are accompanied by myocardial fibrosis. However, myocardial fibrosis is a non-specific finding; it is one of the most common histologic features of the failing heart and has many causes. It may form following myocardial infarction (Sun & Weber 2005; Whittaker et al. 1989), due to hypertension (Díez 2007) or a sequel to inflammatory heart disease (Kania et al. 2009). It is also a common histopathological feature of dilated and hypertrophic cardiomyopathy (Assomull et al. 2006; Ellims et al. 2012; Gulati et al. 2013; Ho et al. 2010; O'Hanlon et al. 2010; Schalla et al. 2010). Its presence alone, therefore, is not indicative of a novel disease nor pathological process which is distinctive to the great ape heart.

3.4.6 Limitations of the study

Since this study was a retrospective observational review, it is limited by many of the same factors as discussed in Chapter 2, including: the authors having little control over raw data collection and recording; there being missing data and information and; great variation in both the approach to examination and descriptive terminology used in reporting diseases between

collections. The issues raised previously with regards retrospective categorisation and based only on the information provided by the zoo, were again highlighted by this study: Most, if not all, deaths are ultimately caused by cardiac failure. However, this is not to say that are caused by cardiovascular disease. Cardiac failure was stated in the post-mortem report as the cause of death for five animals, which were subsequently therefore included in this study. However, each of these animals demonstrated severe renal, hepatic or respiratory pathological lesions, which in fact may have been the inciting cause of systemic organ (i.e. not just cardiac) failure, and therefore their death. These cases therefore demonstrate how, especially in the presence of concurrent cardiac pathological lesions, it is not always possible to definitively determine the exact cause of death.

3.4.7 Implications of the study

The implications of the findings from this retrospective review of great ape cardiovascular disease epidemiology and pathology are many. Not least, they reiterate the need for a full post-mortem examination to be carried out in the event of every great ape death, and for a detailed descriptive report stating not only cause of death but describing all contributing and confounding conditions to be generated. As concluded in Chapter 2, this study again demonstrates the importance of careful categorisation of an animals' cause of death, ideally at the time of death or soon afterwards, taking these findings, as well as clinically relevant information, into account.

Findings related to the typically advanced age at death due to cardiovascular disease, also further substantiates a point already discussed in Chapter 2; that there is a need to further understand and proactively manage chronic and geriatric disease in the ageing zoo-housed great ape population. Nonetheless, the findings also imply that cardiac degeneration is not

merely an inevitable part of great ape ageing, and that reasons for both its occurrence in sub-adult chimpanzees and absence in even aged orangutans, warrant further investigation.

The finding that great ape cardiovascular associated mortalities are frequently sudden or unexpected also implies a need for pro-active ante-mortem screening to identify at risk or affected individuals before it is too late. Given that fibrosis is suggestive of chronic myocardial damage, it is possible that the onset of disease might precede death by months or even years. It is therefore prudent to include even young animals in this screening, not least to gather baseline (healthy) information for future reference. The implementation of routine ante-mortem screening, however, requires sound understanding of which diagnostic tests are of greatest relevance and practical application. The risk of cardiovascular disease related peri-anaesthetic mortality also suggests a need for understanding about the effects of various anaesthetic agents on the great ape cardiovascular system (see Chapter 5) and means by which affected or 'at risk' animals can be identified without the need for anaesthesia. The latter might involve, for example: training animals for conscious echocardiography, electrocardiography; blood pressure assessment; sample (blood, urine) collection or; continual electrocardiogram monitoring using implantable loop recorders.

The study also suggests a need for improvements in the quality of post-mortem examination of the great ape heart, and the consistency with regards approach between zoological collections. It demonstrates a need for the reporting of cardiovascular conditions and lesions to also be improved; for veterinarians' and pathologists' comments relating to subjective and macroscopic findings to be substantiated by the reporting of objective measurements and histopathology findings. Specifically, the study also demonstrates some of the issues associated with the use of the term fibrosing cardiomyopathy as a pathological diagnosis. It implies that there is a need for further characterisation of the fibrosis and accompanying pathological

changes commonly affecting great ape hearts before research striving to identify 'the cause(s) of FCM' in great apes gathers too much momentum.

Finally, this study was able to identify a number of potential risk factors involved in great ape cardiovascular disease epidemiology and pathogenesis, which warrant further exploration. The reasons behind the comparatively lower risk of death due to cardiovascular disease in orangutans, for example, might offer clues as to what it is that makes the other three taxa so susceptible. Also of interest is the increased risk of death due to heart disease among males, and in particular the role that social status, sex and stress, and the complex interplay between them, might play in disease development. It did not, however, assess for any patterns of inheritance nor explore specific genetic factors which might be involved in great ape cardiovascular disease. Given that zoo-housed great ape breeding is tightly managed as part of international breeding programmes, and that cardiovascular disorders such as ARVC with a strong familial basis have been identified by this study and in the literature (Tong et al. 2013), such investigations would be an important next step.

CHAPTER 4:

STANDARDISING THE APPROACH TO THE ANTE-MORTEM AND POST- MORTEM INVESTIGATION OF GREAT APE CARDIOVASCULAR DISEASE

4.1 Introduction

An informal review of existing practices for the investigation of great ape cardiovascular disease across European zoological collections identified several key issues, each of which can limit the progression of understanding in this area (see introductory chapter). One such issue has been the lack of coordination and consistency with regards the clinical (ante-mortem) and post-mortem assessment of cardiac structure and function.

In this chapter, the development of two protocols is outlined:

1. Protocol for the ante-mortem (echocardiographic) assessment of great apes for cardiovascular disease
2. Protocol for the post-mortem examination of the great ape heart

4.2 Ante-mortem (echocardiographic) assessment

4.2.1 Rationale for development of the protocol

Cardiac disease in great apes most commonly manifests as sudden death (Chapter 3) and so most cases are diagnosed only at post-mortem. Pro-active and routine screening is therefore likely to be the only way in which pre- or sub-clinically affected animals can be identified. The tendency within many European collections is for health assessments to be carried out only where a need to do so is otherwise indicated, for example: as part of translocation, for contraceptive implant placement or investigation/treatment of another illness/injury. In many cases, cardiac screening is not routinely included as part of this assessment and, as such, is not a key feature of the zoo's preventative healthcare plan. By comparison, a far greater proportion of great apes housed in zoos across North America are regularly and routinely screened for heart disease as part of routine health assessments, as is recommended by the relevant SSP and their vet advisors (AZA Ape TAG 2010; Clyde 2016; personal communication, Dr H. Murphy, Dr J. Smith).

Given that it is safe, widely available/accessible, and has a wide array of potential clinical applications, transthoracic echocardiography is often considered the mainstay of any such cardiovascular disease screening (Cheitlin et al. 2003). Since 1990, the Great Ape Heart Project (GAHP) have compiled a database of >1200 echocardiographic examinations from 525 great apes housed in zoos, wildlife parks and sanctuaries worldwide. Whilst this database serves as an invaluable resource for the practicing clinician, from a research standpoint there are limitations to the usability of some of the data present within it. The cardiac assessments have been performed by numerous individuals with varying levels of experience and training. The approaches used, as well as the type, number and quality of the echocardiographic images acquired therefore vary. Comparison between these examinations and the quantitative data

gleaned from them is therefore not always possible. It was decided, therefore, that there was a great need for a protocol to standardise ante-mortem cardiac assessment across European zoological collections.

4.2.2 Expectations and aims of the protocol

The purpose of the protocol was to:

- Promote consistency and quality in performing cardiac assessment of great apes between different institutions and by multiple individuals
- Ensure that the same techniques are used when collecting data, thereby allowing comparison between information gathered from various sources
- Facilitate individual clinical assessment and disease screening

It was an ambition that the protocol would be adopted by all professionals performing cardiac assessment of great apes within European zoological collections.

4.2.3 Methodology for protocol development

4.2.3.1 Review of existing protocols

An overview of echocardiographic assessment of structure and function in great apes has been published (Shave et al. 2013). Whilst valuable reading for any clinician embarking upon a great ape echocardiogram in these species, this article was not intended as a protocol for the standardisation of technique or data collection. No such protocols in the field of veterinary medicine were found to exist. There is however, a protocol for a standard adult transthoracic echocardiogram in people, which is published by The British Society of Echocardiography

(Wharton 2012). It provides readers with a minimum dataset to be obtained as well as a recommended sequence for image acquisition. Following consultation with a group of echocardiographic sonographers (Glenfield Hospital, Leicester), it was confirmed that this document is of great use to the day-to-day practicing clinician. The BSE guidelines were therefore selected to form the foundation on which the great ape guidelines under development would be based.

4.2.3.2 Generating a preliminary draft of the protocol

Together with a specialist in veterinary cardiology, the minimum dataset provided in the BSE document was reviewed and those images and measurements to be included in the great ape protocol were selected. The decision about which views/measurements to include/exclude was based upon their predicted clinical usefulness as a measure of cardiac structure and function and the ease at which they could be acquired by the standard clinician.

Unlike people undergoing such assessments, great apes are typically anaesthetised. It is also likely that they will also be undergoing extensive health assessments +/- additional procedures, treatments or translocation during the same anaesthetic. This means therefore that the time available for echocardiographic examination is often limited. For this reason, the list of images to acquire was kept to a minimum.

4.2.3.3 Testing and refining the protocol

The first version of the protocol was followed by a veterinary cardiologist when carrying out echocardiographic examination of three chimpanzees at Twycross Zoo (United Kingdom).

Following its pilot use, the suggested order for image acquisition was altered slightly to improve user-friendliness, and to minimise both the amount of time and turning/re-positioning of the animal required for image acquisition. An animal-side checklist was also added as an aide-memoir, to help users to ensure that all images had been acquired.

Finally, a copy of the protocol was taken to a Great Ape Heart Project (clinical) meeting (Detroit, January 2015) and reviewed by seven cardiac advisors and sonographers, each of whom has significant experience of performing such assessments in great apes. They substantiated its usability and value.

4.2.4 Protocol style and layout

The protocol was intentionally not written in the style of a beginner's guide. The content and wording used is aimed specifically at those individuals who already have a sound understanding of echocardiography in other species. The reason for this was to discourage those without a prior firm baseline skillset in echocardiographic image acquisition from embarking on such assessments, and therefore to limit the number of non-diagnostic quality examinations submitted to the database.

Every effort was made to ensure that, whilst the document was comprehensive in providing sufficient information, it retained its function as a quick reference guide. Where possible the information was conveyed in the form of tables and images. Particular attention was paid to providing example ideal images to be acquired for each acoustic window or measurement, as in the BSE document.

4.2.5 Protocol content

A copy of the complete protocol is provided in Appendix 9.

The opening page of the document contains an introductory section in which the rationale and anticipated purpose of the protocol is provided. It is explained to the reader that following the guidelines provided will not only aid in the clinical assessment of the animal concerned, but also help to contribute towards a growing body of knowledge on this topic.

The section that follows provides some further general guidance for the reader, including a list of information to be gathered at the time of examination, such as: the animal's identifying information, species, date of birth/age, holding zoological collection, weight, body measurements, current health status +/- any medication(s) the patient is on; examination date and sonographer details. It also provides readers with guidance for data storage and subsequent submission of the scan to the Great Ape Heart Project database.

The protocol is written with the assumption that the echocardiogram is being carried out in animals which are anaesthetised. Given that understanding about the ways in which various anaesthetic agents affect the great ape heart and especially the echocardiogram is still limited (see Chapter 5), no recommendations about the drugs to use/not to use were included in the protocol. However, in order to allow for echocardiographic findings to be interpreted in the context of the anaesthetic protocol used, those performing the examination are asked to provide detailed information regarding the dose and timing of any drugs administered during the procedure as well as the animal's blood pressure and heart rate at the time of the examination.

Also included in the protocol are the following: technical guidance relating to the equipment required and recommended settings; suggested position (and accompanying demonstrative image) of the animal; guidance relating to patient skin preparation and; details regarding

acoustic window location. Finally, the protocol also includes a brief information about concurrent data and sample collection, including blood pressure, electrocardiogram (ECG), cardiac biomarker assessment, and serum/blood samples for future analysis.

The majority of the remaining document is dedicated to the minimum dataset of images and measurements to be gathered as part of a standard great ape echocardiographic examination. As for the document generated by the BSE, the images acquired when following this protocol are intended a minimum dataset only, meaning that they should be supplemented with additional images as indicated by the presence of pathological lesions. Similarly, not all images included would be acquired as part of follow up studies or in targeted studies carried out for example, when checking for a pericardial effusion.

4.2.6 Follow up

After performing the cardiac assessment, the individual performing the scan or the zoo themselves are asked to submit it to the GAHP (USA). For European zoos specifically, the images are then reviewed by the European project's lead cardiac advisor (Royal College of Veterinary Surgeons recognised specialist in veterinary cardiology) and a GAHP cardiac advisor (medical or veterinary cardiologist with experience assessing great ape hearts). Two types of feedback are then given to the zoo: 1) An opinion on the clinical status (structure and function) of the heart, and: 2) A comment on image quality for data integrity purposes. Only those examinations which are deemed to be of adequate quality will go on to be included in any future research studies. For those not of adequate quality, feedback is given on how the examinations can be improved in the future. Ongoing support or advice is also available.

4.3 Post-mortem assessment

4.3.1 Rationale for development of the protocol

Whilst it is an expectation that zoological collections will arrange for a full post-mortem examination to be carried out on any great ape that dies, there is currently no stipulated requirement nor guidelines for how to do this in Europe. As a result, post-mortem examinations are not carried out in every case and, when they are, there is great variation in both the examination itself and the subsequent recording of findings (Chapter 2). Specifically, macroscopic and histopathological examination of the heart is not performed in every great ape death. When conducted, there is inconsistency with regards to the: expertise of the individual performing the examination; extent of the examination; samples examined; terminology applied, and quality and quantity of information provided in the report that follows (Chapters 2 & 3). This limits not only the diagnostic power of the examination but also the potential for meaningful comparison between the findings for multiple animals, and therefore the scope for large scale multi-centre and longitudinal studies to be carried out. Additionally, there is a lack of availability of comparable, good quality samples for research, which results in competition for resources and again, limits the potential for further research into great ape cardiovascular disease aetiology and pathogenesis.

For these reasons, it was decided that there was a great need for a protocol to standardise the approach to the great ape cardiac post-mortem examination, data and sample collection across European zoological collections.

4.3.2 Expectations and aims of the protocol

The purpose of developing the protocol was to:

- Promote consistency and quality in performing post-mortem examination of the great ape heart
- Standardise and maximise information gathering
- Apply a consistent and gold standard examination to great ape hearts housed in multiple collections
- Collect consistent good quality biological samples from numerous collections

The aim was for the information, data and samples collected through implementation and use of this protocol to:

- Allow detailed characterisation of the pathological lesions found to affect the great ape cardiovascular system using a repeatable system and consistent terminology
- Facilitate comparative study between post-mortem examination findings and relevant biological samples collected from numerous institutions

It was an ambition that all individuals working within a zoo or sanctuary would follow the protocol in the instance of a great ape death in their collection.

4.3.3 Methodology for protocol development

4.3.3.1 Review of existing post-mortem protocols

At the time of commencing protocol development, there were no available guidelines for the examination of great ape hearts. Suggested processes for post-mortem examination of the heart are included in numerous textbooks of veterinary pathology. However, these have largely been written with the intent of helping the examiner to identify conditions which commonly affected domestic animal species and so their relevance to great ape cardiovascular disease is limited. It is likely, instead, that the anatomy and physiology, and perhaps also therefore the pathology of the great ape heart is more akin to that of humans than for example dogs, cats or livestock. Additionally, deaths due to cardiac disease in domestic species are most frequently not sudden or unexpected: whether natural or due to euthanasia, the death most frequently follows a period of time during which the animals display clinical signs of illness. In contrast, great ape cardiac disease is frequently associated with death with no prior clinical signs. For the individual performing the examination, the situation is therefore most comparable to the investigation of sudden or unexplained death in humans.

A review of the (human) medical literature identified two papers of particular interest and relevance. The first (Basso et al. 2008) was compiled by numerous authors on behalf of the Association for European Cardiovascular Pathology. Like the BSE guidelines for echocardiographic examination discussed in Section 4.2 above, it serves as a minimum standard for those performing post-mortem examinations to follow. As for the great ape protocol under development, it too aims to promote a uniform method of investigation across numerous European institutions. The paper provides readers with a broad overview of the information to be gathered regarding the death and steps to follow for the exclusion of non-cardiac causes of death, as well as gross and histopathological examination of the heart. The

second paper identified (Sheppard 2012) focuses on the heart specifically and outlines one expert's process for the dissection and examination at the heart at autopsy. Through a combination of words, diagrams and images, it provides a more detailed and comprehensive process for each individual part of the cardiac examination. Based upon their relevance, easy to follow style, comprehensive coverage and inclusion of photographs and diagrams, these two papers were selected to form the foundations on which the great ape cardiac post-mortem examination protocol would be based.

4.3.3.2 Generating a preliminary draft of the protocol

Together with a veterinary pathologist, both papers were carefully reviewed and those sections to be included/excluded in the great ape protocol selected. The decision about which areas to include/exclude was based upon their relevance to the intended aims of the protocol and to great apes, and the ease with which such information, data or samples could be collected and recorded by the individual performing the examination.

Unlike the situation in humans whereby a trained coroner is responsible for carrying out the complete autopsy, individuals carrying out such an examination on great apes may simply be veterinarians with little or no pathological examination experience or even trained keeping or curatorial staff. It was therefore decided that the protocol would have two distinct parts: Part 1 would consist of information gathering and basic examination of the heart, major vessels and thoracic cavity, usually to be performed at the zoo itself. It would outline the steps to follow for the consistent sampling and fixing of the heart. The anticipated end point for part 1 would be the submission of the heart to the University of Nottingham for more detailed examination.

Part 2 would be the protocol for a more detailed gross examination and subsequent trimming of the heart, to be carried out at the University of Nottingham. The anticipated end point for this protocol would therefore be the collection of research data and the issuing of a report relating to the main findings of the gross and histological examination.

4.3.3.3 Testing and refining the protocol

Both parts of the protocol were reviewed by Professor Mary Sheppard; a cardiovascular pathologist, expert in sudden cardiac death in humans, and author/co-author of the two papers on which the protocol was being based. Her feedback was used to further enhance the protocol's completeness. Further consultation with researchers working at the University of Nottingham (School of Veterinary Medicine and Science) within the fields of pathology, clinical cardiology, molecular biology and infectious disease was also undertaken. Following this, a list of biological samples of use and interest for future research was generated and instructions for their collection added to the protocol.

Together with veterinarians at Twycross Zoo, Part 1 of the protocol was then tested as part of the post-mortem examination of one great ape and one non-great ape primate. Based upon the feedback obtained through this process, the following changes were made:

- The wording of some of the stages was changed to further clarify the process. This is particularly true of the stage involving removal of the heart from the pluck (Step 4, Appendix 10)
- The order of the stages was adjusted slightly to make the protocol simpler and more time efficient to follow
- The wording was condensed to make the protocol more concise

- A photograph demonstrating the location and direction of the incision to be made across the heart apex was added

Together with a veterinary pathologist, Part 2 of the protocol was then tested on three great ape hearts, and several minor additions/changes made to further streamline and clarify the process.

Over time, following increased use, minor adaptations to the protocol were made to further improve its completeness and user-friendliness. These changes were mostly to Part 2 of the protocol, and were based upon progression in knowledge, gleaned through ongoing experience of using the protocol for clinical and research information gathering purposes. Minor changes to part 1 of the protocol have been minimal and involved clarification of the process for sample submission.

The final stage of protocol refinement was to use the protocol to demonstrate and teach the examination process to a new, additional observer. Based upon the feedback gained through this process, further prompting questions and comments relating to common normal versus abnormal findings, as well as explanatory photographs, were added.

4.3.4 Protocol style and layout

4.3.4.1 Part 1: basic examination, sampling and fixation

In most instances, part 1 of the protocol would be carried out at the zoological collection or sanctuary where the great ape had died. Although in some instances, a trained pathologist would be responsible for the examination, it would be more common for the protocol to be carried out by zoo or sanctuary's veterinary, keeping or curatorial staff. The wording would

therefore need to be appropriate for the level of training/expertise those performing the examination are likely to have, i.e. not assume too much prior experience in this area nor consist of too much technical jargon.

In the event of a great ape death, there are also a lot of other matters for these individuals to consider, such as: examining the rest of the carcass; identifying the cause of death (if possible); managing the health and social stability of other animals in the group; communication with staff, public and the press and; the collection of samples and data for other research projects or interested parties. The accuracy and consistency with which the protocol is followed, therefore, is likely to be depend highly upon how easy and time consuming it is to follow. The protocol was therefore designed to be as simple as possible to follow and to fit on one side of A4 so that it could be laminated and displayed, for example, on the wall of the veterinary clinic or post-mortem examination room.

Where possible, the sections and style of writing used was kept as similar to that of the guidelines for ante-mortem assessment as possible, in order to ensure consistency and promote familiarity for the reader.

4.3.4.2 Part 2: detailed examination of the formalin fixed heart

Unlike part 1, part 2 of the examination would be carried out by only two individuals, both of whom have additional training and experience in pathology and were themselves responsible for protocol development. The need to keep the number of steps to a minimum and to avoid technical jargon, therefore, was far less. Nonetheless, the instructions included needed to be clear to promote consistency and thereby reduce inter-observer variability. The inclusion of photographs and diagrams also helped to clarify where required. The protocol was designed to

provide not only a comprehensive guide as to all stages of examination, but also a suggested order to follow. It was intended to be used as a reference guide for the observer to use during the examination.

4.3.5 Protocol content

4.3.5.1 Part 1: basic examination, sampling and fixation

A copy of Part 1 of the protocol is provided as Appendix 10.

The opening page of the document contains an introductory section in which the rationale and anticipated purpose of the protocol is provided. Readers are asked to ensure that photographs are taken at all stages of the examination process, and that, should any abnormal fluid accumulation be identified, that it is quantified (in ml or g, if clotted) and characterized (colour, consistency, specific gravity), and a sample stored where possible.

The following section provides some further general guidance, including a list of information to be gathered at the time of examination, such as: animal identification information, species, holding zoological collection, date of birth/age and date of death. Readers are prompted to ensure such information is also displayed on all submitted samples and paperwork. They are also asked to supply a copy of the full post-mortem report along with a copy of the following where possible: the animal's clinical history, the animal's breeding/holding records, photographs taken during the post-mortem examination. Additional information about sending the samples including reference to CITES regulations is also provided.

The page which follows is intended to be used as a reference guide that can be laminated and displayed, for example on the wall of the post-mortem room. It consists of a table within which the eight-step guide for inspection and sampling of the heart is provided. The remaining five

steps within the table are dedicated to the subsequent formalin fixation of the heart and shipment of the samples to the University of Nottingham for more detailed examination. Also provided is a reminder to take photographs, and contact details should they require any additional information or support. Overleaf a figure demonstrates the location of the transverse cut which is to be made across the lower third of the heart apex (see Step 6, Appendix II).

4.3.5.2 Part 2: detailed examination of the formalin fixed heart

A copy of Part 2 of the protocol is provided as Appendix 11.

Provided on the first page of this protocol is a list of information for the examiner to ensure that have got prior to commencing the examination. This information not only ensures accurate labelling of any samples taken but also helps with interpretation of the findings. It includes: animal identification, species, sex, date of birth/age and date of death, zoological collection as provided in Part 1. Additional information also required is: a list of samples submitted, a summary of the post-mortem examination findings, history and circumstances +/- cause of death and heart weight (fresh, pre-fixation). Also provided is a list of the equipment required to carry out the examination and a reminder of the labelling system to be used for all samples taken as part of the examination process.

Thereafter, detailed instructions including prompting questions and additional comments regarding normal versus abnormal findings are given. The stages include: assessment of the sample condition; amount of fat; and quality of preservation; a morphological description of shape, colour and any lesions evident on the epicardium; heart weight; wall thickness measurements; coronary vasculature sectioning and examination; major vessel, valve and chamber examination; myocardial sectioning and examination and; sampling for histopathology.

Alongside the protocol, a data collection form was also developed. It was designed to follow the same sequence as the examination and to not only therefore aid the examiner in recording findings, but to also serve as a checklist of information and data to be gathered. A copy of this data collection form is provided as Appendix 12.

4.3.6 Follow up

The end-point of the protocol is the submission of samples for histopathology. All tissue sections were then processed, sectioned and stained (haematoxylin and eosin stain). The slides were then examined under the supervision of a boarded pathologist and qualitative descriptions relating to the findings on each one were added to the data collection form. Histological diagnoses were generated and interpreted alongside the findings of the macroscopic examination. These were combined into a final report, which was issued to the zoological collection submitting the sample. The report details the macroscopic and histopathologic findings and includes a final interpretive comment. It provides much greater detail than the standard reports typically provided by most veterinary pathologists. The interpretation given is also based upon other similar cases presented by other collections.

4.4 Protocol dissemination

Once complete, both protocols were uploaded to the Ape Heart Project webpage (part of the Twycross Zoo website), where they would be freely available for anyone wishing to access them.

Awareness about the protocols was then raised at the annual European Association for Zoo and Wildlife Veterinarians conference in Barcelona, in May 2015. A verbal presentation was given by means of an introduction to the European project and protocols, and a meet-and-greet session followed. As part of this session, information packs were issued to any vets working within great ape holding zoos who were interested in participating in the project. Both protocols (including laminated copies of both the echo checklist and post-mortem examination/sampling instructions) were included in this pack.

An additional twenty zoological collections, selected based on the number of great apes in their collection, were also sent a copy of these information packs via the post. They were also issued with a sampling kit (see Figures 4.1a, 4.1b). These kits contained the equipment required for basic examination and sampling as per the protocol. They were packaged within a plastic lidded container which could also be used for formalin fixing +/- shipping the heart.

The use of the protocols has also been endorsed by the EAZA great ape Taxon Advisory Group: the vet advisors and species coordinators recommend that all great apes undergo cardiac screening as part of routine health check procedures and ask that, in the event of any great ape death, the heart is examined, sampled and submitted as per the post-mortem protocol. These recommendations have also been incorporated into the Veterinary Section of the most recent Best Practice Guidelines (in draft).

Figures 4.1a&b: Image showing contents of sampling kit including: zip-lock bags for enclosing frozen tissue samples; universal tubes for storage of frozen/liquid samples; 1ml vials containing RNAlater; labels required for sample shipment; scale marker and label for photographs; gauze swabs for wrapping heart sample (**Figure 4.1a, left**) and; plastic lidded container **(Figure 4.1b, right)** within which the above was contained

4.5 Discussion

The aim of the protocols and overall purpose for their development was to standardise and improve the quality of the current approach to great ape cardiovascular disease investigation across European zoological collections. The anticipated goals were for consistent data, information and samples gathered from numerous sources, to be used not only for the clinical or pathological benefit of the individual animals and zoological collection concerned, but ultimately to facilitate multi-centre comparative studies investigating great ape cardiovascular pathogenesis. As outlined in section 4.4, the protocols which have been developed meet the aims and objectives initially set out.

The relative merits and limitations of the post-mortem protocol and the data generated through its use, will be covered in Chapter 6. However, the echocardiography protocol and indeed also the use of echocardiography as a diagnostic tool in great ape cardiovascular disease is discussed below:

The first topic to consider is the limited understanding of "normal" versus "abnormal" echocardiographic findings that currently exists for great apes. Echocardiographic parameters from clinically normal gorillas (Murphy et al. 2011) and chimpanzees (Sleeper et al. 2014) have been published, although these papers are not without limitation: the study populations used were relatively small and not all sub-groups (e.g. age categories) were represented in the dataset. Additionally, the animals were anaesthetised, which is necessary for safety reasons and is therefore largely unavoidable. Although the effects of various anaesthetic agents on the usefulness of echocardiography as a diagnostic tool are often debated (see Chapter 5), it cannot be disputed that most, if not all, anaesthetic agents will have some effect (whether direct or indirect) on the cardiovascular system. Therefore, any echocardiogram carried out in an anesthetised animal must be interpreted with caution, and factors such as the individual drugs

administered, the time since administration and concurrent haemodynamic factors (e.g. heart rate and blood pressure) should also be considered.

It is also prudent to suggest that understanding about the echocardiographic appearance of great ape cardiovascular disease is, at present, very limited. In humans and many domestic animal species, the clinical diagnostic criteria for commonly diagnosed conditions have been well defined. This does, however, not hold true for any of the great ape species. For some conditions, such as structural or anatomical deformities, extrapolating what is known from human or domestic animal species is most likely sufficient, appropriate and relatively easy for the experienced clinician to do. This is especially true where the extent of the pathological lesions is particularly advanced or severe. It is less simple, however, in the subtler of cases, especially in the absence of normal reference ranges. For example, when trying to determine if a left ventricular wall which appears thickened on an echocardiogram is genuinely thickened as the result of systemic hypertension, an infiltrative or hypertrophic cardiomyopathy, or whether it falsely appears so due to impaired left ventricular filling in an anaesthetised, recumbent animal. Even more complicated is the situation with regards fibrosing cardiomyopathy/idiopathic myocardial fibrosis; a pathological description which has been reported widely across the great ape literature to be associated with peri-anesthetic death, congestive heart failure, cerebrovascular infarction, and most commonly, sudden or unexpected death (Chiu & Bodley 2010; Lammey, Lee, et al. 2008; Miller et al. 1999; Munson & Montali 1990; Schulman et al. 1995). Various pathological descriptions exist, but they typically cite an increase in fibrous tissue within the myocardium, with no obvious evidence for an underlying aetiology (see Chapters 3 & 6). As yet, it is not known how this condition can be diagnosed ante-mortem, for example, does it cause: hypertrophy and/or dilation of affected chambers; progressive systolic or diastolic dysfunction and heart failure and/or; terminal arrhythmias? If the latter is true, it might be the case that electrocardiography is in fact of greater diagnostic use than

echocardiography. Answering these questions and many others is not yet possible, and will require a great deal of further research, specifically longitudinal monitoring of a large cohort of animals and correlation of both ante-mortem and post-mortem findings with one another.

It must also be remembered, that the most comprehensive and accurate echocardiogram, even if not performed under the influence of anaesthesia, provides only a snapshot image of heart structure and function at any given time-point. The cardiovascular system and its interplay with systemic endocrinological, metabolic and haemodynamic factors is complex and dynamic. Those interpreting the findings of cardiac assessments must therefore bear this in mind, and consider the potential for employing additional, more regular or longer term methods for assessing and monitoring cardiac structure and function. Whilst outside the remit of this discussion, it is prudent to mention the role that, for example, serum cardiac biomarkers, blood pressure assessment or continual electrocardiogram monitoring (using loop recorders) might therefore play in great ape cardiovascular disease diagnosis.

Finally, it must be caveated that implementation of the guidelines does not guarantee an excellent quality, diagnostic scan of the cardiovascular system. Even in the hands of an experienced operator, there remain several factors which might impede or limit image acquisition, for example: the animals' size or bodyweight, the size and extent of their air sacs (especially in adult male orangutans), the presence of lung disease or operator inexperience. This demonstrates the importance of the review and feedback process outlined in section 4.2.6, especially when selecting those examinations which are to be included in the dataset for future research studies. The repeatability and inter-observer reliability of this protocol's ability to generate research data have, however, not yet been tested. Statistical validation of the methodology recommended by this protocol for gathering such data, would therefore be an interesting next step for research in this area.

4.5.1 Future use and development of protocols

Neither protocol is designed to be a final nor definitive guide. Each will need developing and updating as knowledge and understanding progresses, and will therefore evolve and adapt over time. Yet further improvements and refinements might also be made by incorporating the opinions of additional experts working within the relevant fields: a questionnaire based method such as the Delphi technique might be most suitable for this (Grime et al. 2016).

It is hoped that the implementation of these protocols will not only improve the quality of both ante- and post-mortem investigations of cardiovascular disease performed in great apes across Europe, but also generate a rich source of valuable data and samples for further research in this field. Any such research has the potential to ultimately improve European and global captive great ape health and welfare.

CHAPTER 5:

COMPARISON OF THE CARDIOVASCULAR EFFECTS OF TWO DIFFERENT ANAESTHETIC PROTOCOLS IN CHIMPANZEES

5.1 Introduction

The topic of cardiovascular disease among zoo-housed great apes has predominated the zoological literature in recent years (Strong et al. 2016; Chapter 1) and has been identified as a major cause of mortality across all of the great ape taxa (Chapter 2). Affected animals often do not display clinical signs of disease and present only as cases of sudden (unexpected) death (Chapter 3). For this reason, pro-active screening is key to identifying affected animals early in the disease process. However, the size and potentially dangerous nature of the animals in question require that most such cardiac assessments are carried out in immobilised/anaesthetised animals.

Various drug combinations can be used in the sedation, immobilistion and/or anaesthesia of great apes (Cerveny & Sleeman 2014). The alpha-2-agonists and in particular, medetomidine, is used very frequently because, in combination with other drugs such as ketamine or tiletamine/zolazepam, it offers rapid induction; safe, stable reliable and reversible sedation/immobilization; excellent muscle relaxation and; calm and smooth recoveries (Adami et al. 2012; Lewis 1993; Naples et al. 2010). However, its use remains controversial given anecdotal reports that it not only impedes meaningful interpretation of echocardiographic findings but to also increase the risk of (peri-)anaesthetic mortality (Brainard 2016; GAHP 2016a).

Medetomidine binds to alpha-adrenergic receptors in the peripheral vasculature, causing vasoconstriction and an increase in systemic vascular resistance. The initial effect of this is an increase in blood pressure, but the baroreceptor response typically then results in slowing of the heart rate and a reduction in cardiac output (and ultimately blood pressure). A variety of studies investigating the effects of alpha-2-agonists specifically on echocardiographic parameters have been studied in rats, dogs and in humans (Baumgartner et al. 2010; Dodam et

al. 2004; Kallio et al. 1990; Rand et al. 1996; Romagnoli et al. 2016; Sabatini et al. 2013; Saponaro et al. 2013). The conclusions of these studies are, however, variable and sometimes contradictory. Many of the studies have also been carried out under tightly controlled settings, often making use of invasive monitoring techniques to measure the haemodynamic response to drug administration. The degree to which the findings can be applied to the field anaesthesia of great apes in a zoological setting is therefore uncertain.

There is only one published review in which the echocardiographic effects of various anaesthetic agents on echocardiographic parameters in great apes were studied: Napier et al. (2013) evaluated and compared echocardiographic parameters and blood pressure measurements of seven gorillas during three stages of an anaesthetic protocol. They concluded that medetomidine is associated with a significant reduction in ejection fraction and increased left ventricular dimension and suggested therefore that caution be used when assessing cardiac function in animals exposed to medetomidine. There are, however, many limitations to this study which bring the validity of their conclusion into question. Firstly, all gorillas studied were adult males, a sub-population among which cardiovascular disease is known to be particularly prevalent (Meehan & Lowenstine 1994; Murphy et al. 2011). Five of the seven animals under study did in fact have evidence of cardiovascular disease. Given that the diseased heart is likely to respond differently to anaesthesia when compared with a healthy heart (Moyna 2012), the findings of this study may not be relevant to the wider (healthy) population. In addition, the study followed the same animal throughout one procedure: phase 1 data being collected when the animals had received only medetomidine and ketamine; phase 2 being after the addition of sevoflurane; and phase 3 data being collected after the reversal of medetomidine with atipamezole. Due to the sequential nature of their data collection within one procedure, the authors were not able to confirm that the differences were observed were definitely the result of the addition or removal of medetomidine and not some other variable.

The aim of this study therefore was to compare the effects of two different anaesthetic protocols (tiletamine/zolazepam +/- medetomidine) and specifically to evaluate their suitability for the immobilisation of healthy chimpanzees undergoing assessment of cardiac structure and function.

5.2 Materials and methods

The study was performed with the approval of the ethical review committee of the University of Nottingham's School of Veterinary Medicine and Science (February 2016; 1692 160222).

5.2.1 Animals

Two male and four female chimpanzees (*Pan troglodytes*) housed at Kolmården Wildlife Park, Sweden were the subject of this study. The animals were part of a mixed age, mixed sex group (n=25). They were housed in an indoor/outdoor enclosure, had free access to drinking water, and were fed a mixed diet consisting of vegetables, fruit, branches and a commercially available pellet based product. The animals were being moved to another zoological collection, for which they underwent two anaesthetic procedures less than 8 weeks apart: 1) To perform a pre-export health assessment; and 2) To confirm their good health status and allow safe transfer into a crate for transport on the day of export.

5.2.2 Experimental design

The study was carried out as a prospective blinded clinical cross over study. The procedures and data collection/recording were carried out by the same team of individuals on both occasions. Those recording the data were blinded as to the hypotheses of the effects of the two different anaesthetic protocols being used. Food, but not water, was withheld for >6 hours prior to the procedures being carried out.

5.2.2.1 Anaesthetic protocol

On the morning of the first procedure only, all animals were offered midazolam, orally. All animals were administered the anaesthethic induction agent intramuscularly (IM) by blow dart. Estimated body weights were used for calculating induction dosages. For procedure 1, only tiletamine-zolazepam (Zoletil 100 Vet., Virbac, France; 3-4 mg/kg) was administered for the induction of anaesthesia. For the second procedure, a combination of tiletamine-zolazepam (2 mg/kg) and medetomidine (Zalopine, Orion Pharma, Finland; 0.02mg/kg) was used. Supplemental doses of ketamine (Ketaminol vet., Intervet, The Netherlands) +/- tiletamine-zolazepam were administered IM/IV as required, for example if arousal was observed.

Once recumbency was achieved and an absence of voluntary movements or lack of response to stimuli was observed, the animals were removed from their enclosure and moved to an adjacent examination room where they were weighed and then placed into dorsal recumbency. Oxygen was delivered via endotracheal or nasal tube.

5.2.2.2 Monitoring

Monitoring commenced once the animals were on the examination table. All chimpanzees were monitored continuously and parameters recorded at 5 minute intervals. Clinical criteria used to assess the plane and stability of anaesthesia included: respiratory rate (f_R), eye position, palpebral reflex, jaw tone, muscle relaxation and spontaneous movement. Heart rate (HR) was measured by auscultation and pulse rate (PR) via digital palpation of the femoral artery. Arterial oxygen saturation (SpO_2, %) was measured using a pulse oximeter with the probe placed on the lip/tongue. Systolic (SAP), mean (MAP) and diastolic (DAP) blood pressure were measured using a non-invasive oscillometric device (AEG, BMG 4907, Germany; Omron R1, OMRON Healthcare GmbH, Germany) with an appropriately sized cuff placed on the arm.

5.2.2.3 Echocardiography

A detailed echocardiogram (Vivid q ultrasound system, GE Healthcare, Hatfield, United Kingdom) was performed with the animal in left dorso-lateral recumbency as per the protocol issued for use in European zoos (Strong et al. unpublished; Chapter 4 Appendix 9). The following echocardiographic measurements were collected: left atrial diameter (LA), aortic root diameter (Ao), interventricular septum thickness in diastole (IVSd), left ventricular end diastolic diameter (LVIDd), left ventricular end systolic diameter (LVIDs), left ventricular posterior wall thickness (LVPW), ejection fraction (EF; by Simpson's biplane), left ventricular outflow tract diameter (LVOT), peak flow velocity at the pulmonic valve (Vel_{PV}) and aortic valve (Vel_{AV}), tissue (E and A wave) velocities (E' vel., A' vel.).

Echocardiographic image acquisition and interpretation were performed by the same individual on both occasions and later reviewed offline by a specialist in veterinary cardiology to validate the interpretations.

5.2.2.4 Additional procedures

Routine health assessment was performed during both procedures and consisted of clinical examination (including dental examination) and blood sampling for routine haematology and biochemistry. During the first procedure only, the following were also conducted: a 12-channel electrocardiogram (CardioExpress SL6, Spacelabs Healthcare Ltd., United Kingdom); blood sampling for serum cardiac biomarker (P-Troponin T hs & P-NT-Pro BNP) assessment and serological screening (results not presented) and; comparative intra-palpebral tuberculin testing. Additional medical/surgical interventions were also carried out as required for each animal.

5.2.2.5 Anaesthetic scoring and timing

The animal's pre-anaesthetic (post-midazolam) activity level and demeanour at the point of induction were scored using a basic descriptive scale devised and adapted by the observers from studies carried out in other species (Ferreira et al. 2015; Kerr et al. 1996). The delivery success was recorded as a percentage of the total dose administered (complete: 100%). The time taken to achieve a light plane of anaesthesia (defined as recumbency, muscle relaxation, and an absence of voluntary movements) after administration of the induction agent was recorded in minutes. The overall quality of the anaesthetic was quantified based upon the combined scores given for: quality of induction (1: excellent to 4: poor); degree of muscle relaxation (1: excellent to 4: poor) and; ease of intubation (1: easy to 4: extremely difficult). All used a four-point scale in which a lower score indicated the favourable outcome. The plane of anaesthesia achieved following administration of the induction agent +/- any supplemental doses was also scored. Descriptions of all scoring criteria used are provided in Appendix 13.

5.2.2.6 Anaesthetic recovery

At the end of the procedure, animals were moved from the examination table to their enclosure (TZ) or transport crate (TZM). Following the second procedure (TZM), the medetomidine was antagonised by the intramuscular administration of atipamezole at a dose of five times that of the medetomidine. The timing of atipamezole administration relative to the end of the procedure varied between chimpanzees and was influenced by several factors, for example the need to mix and group animals for transport. All animals were monitored closely throughout recovery and extubated when an adequate swallow reflex for airway protection was observed. The quality of anaesthetic recovery was scored using criteria used for dogs previously (Kennedy & Smith 2015) (Appendix 13).

5.2.3 Statistical analysis

Statistical analysis was carried out using GraphPad Prism 7 for MacOS X (2016). Data were assessed for normality using the Shapiro-Wilk test. Parametric data were compared using paired Students t-tests; the Wilcoxon matched pairs test was used for non-parametric data. Data are presented as mean ± SD or median (min; max). Differences were considered statistically significant when $p<0.05$. Confidence intervals were set at 95%.

5.3 Results

Demographic data relating to the study population are provided in Table 5.1.

5.3.1 Health assessment

All animals were found to be in good overall health with no evidence of serious illness detected following clinical examination, comparative palpebral tuberculin testing or routine haematology and biochemistry. No abnormalities were detected on cardiac auscultation nor electrocardiogram and all animals' cardiac biomarker results were within the normal reference ranges published for use in humans (no such ranges for chimpanzees exist).

5.3.2 Haemodynamic and respiratory parameters

Data relating to all haemodynamic and respiratory parameters for both anaesthetic protocols are displayed in Table 5.2. The TZM anaesthetic protocol was associated with a significant reduction in heart rate (Figure 5.1) and pulse rate.

TZM anaesthesia was also associated with lower systolic, diastolic and mean arterial blood pressures than TZ anaesthesia (Figure 5.2). The mean SAP/DAP/MAP measurements obtained (Table 5.2) during TZM anaesthesia were within the normal limits for humans (no such published reference ranges for chimpanzees exist). However, two consecutive individual MAP readings of <60mmHg (hypotensive) were obtained from one animal (Chimpanzee 2). The mean SAP/DAP/MAP measurements obtained (Table 5.2) during the TZ procedure exceeded normal (human) blood pressure ranges.

Table 5.1 Table showing study population demographic information and data relating to anaesthetic induction, maintenance and recovery. *Abbreviations: M: medetomidine; K: ketamine; ND: no data collected/recorded; NSF: no significant findings; TZ: tiletamine-zolazepam administered for induction of anaesthesia; TZM: tiletamine-zolzepam and medetomidine administered for induction of anaesthesia.*

		Chimpanzee 1		**Chimpanzee 2**		**Chimpanzee 3**		**Chimpanzee 4**		**Chimpanzee 5**		**Chimpanzee 6**	
		TZ	***TZM***	***TZ***	***TZM***	***TZ***	***TZM***	***TZ***	***TZM***	***TZ***	***TZM***	***TZ***	***TZM***
GENERAL	***Sex***	Male		Female		Male		Female		Female		Female	
	Age *(at 1st procedure)*	16 years 4 months		15 years 4 months		5 years 5 months		16 years 6 months		14 years 7 months		4 years 7 months	
	Est. bodyweight *(kg)*	60	60	50	50	20	25	85	80	55	50	20	25
	Actual bodyweight *(kg)*	60	60	47.5	ND	19.5	22.5	78.5	78	47	ND	21	23.5
PRE ANAES-THETIC	***Starve period*** *(hrs)*	2-8	16-24	2-8	16-24	2-8	16-24	2-8	16-24	16-24	16-24	16-24	16-24
	Midazolam: *dose (mg); success (%)*	60; 0	None	50; 100	None	20; 0	None	60; 90	None	55; 80	None	20; 100	None
INDUCTION	***Anaesthetic induction:*** *drug; dose (mg)*	240 TZ	120 TZ; 1.2 M	150 TZ	100 TZ; 1 M	60 TZ	50 TZ; 0.5 M	300 TZ	160 TZ; 1.6 M	220 TZ	100 TZ; 1 M	70 TZ	50 TZ; 0.5 M
	Dose for est. bodyweight *(mg/kg)*	4 TZ	2 TZ; 0.02 M	3 TZ	2 TZ; 0.02 M	3 TZ	2 TZ; 0.02 M	3.53 TZ	2 TZ; 0.02 M	4 TZ	2 TZ; 0.02 M	3.5 TZ	2 TZ; 0.02 M
	Dose for actual bodyweight *(mg/kg)*	4 TZ	2 TZ; 0.92 M	3.16 TZ	ND	3.08 TZ	2.22 TZ; 0.22 M	3.82 TZ	2.05 TZ; 0.02 M	4.68 TZ	ND	4.68 TZ	2.13 TZ; 0.02 M
	Administration success *(%)*	100	100	30-40	100	100	100	100	100	100	100	100	100
	Repeat darting: *drug, dose*	None	None	70 K; 35 K	None	None	None	None	None	None	None	None	None
	Time to light anaesthesia *(min)*	1.5	5	21	4	4	5	9	5	4	4	5	3
MAINTEN-ANCE	***Supplemental doses:*** *n; total (mg)*	3; 140 K	0	2; 55K	0	7; 175 K	0	5; 280 K;60 TZ	0	3; 150 K	0	4; 80K, 20 TZ	0
TIME: INDUCTION TO ECHO *(mins)*		42	40	58	17	75	37	32	29	41	23	45	30

Table 5.2 Shows haemodynamic, respiratory and echocardiography data for both anaesthetic protocols (TZ and TZM)

Variable		**TZ**	**TZM**
Haemodynamic and respiratory parameters	HR *(bpm)*	87 ± 3	62 ± 6****[1]
	PR *(bpm)*	86 ± 10	70 ± 3****[1]
	SAP *(mmHg)*	150 ± 18	116 ± 9**[1]
	DAP *(mmHg)*	92 ± 4	69 ± 8**[1]
	MAP *(mmHg)*	114 ± 6	85 ± 8**[1]
	SpO_2 *(%)*	97 ± 1	93 ± 2****[1]
	f_R *(bpm)*	19 ± 3	16 ± 3
Echocardiography measurements	Ao *(mm)*	20 ± 2	22 ± 4
	LA *(mm)*	30 ± 6	30 ± 6
	IVSd *(mm)*	8 ± 1	9 ± 2
	LVIDd *(mm)*	44 ± 4	41 ± 6
	LVIDs *(mm)*	28 ± 2	27 ± 4
	LVPW *(mm)*	7 ±1	6 (6; 10)
	EF *(%)*	56 ± 4	56 ± 5
	LVOT *(mm)*	17 ± 3	17 ± 3
	Vel_{PV} *(m/s)*	1 ± 0.16	0.8 (0.6; 0.8)
	Vel_{AV} *(m/s)*	0.4 (0.1; 0.6)	1 ± 0.
	E' vel. *(m/s)*	0.8 ± 0.2	0.8 ± 0.2
	A' vel. *(m/s)*	0.5 ± 0.2	0.4 ± 0.1

[1] ***: p ≤ 0.01; ****: p ≤ 0.0001*

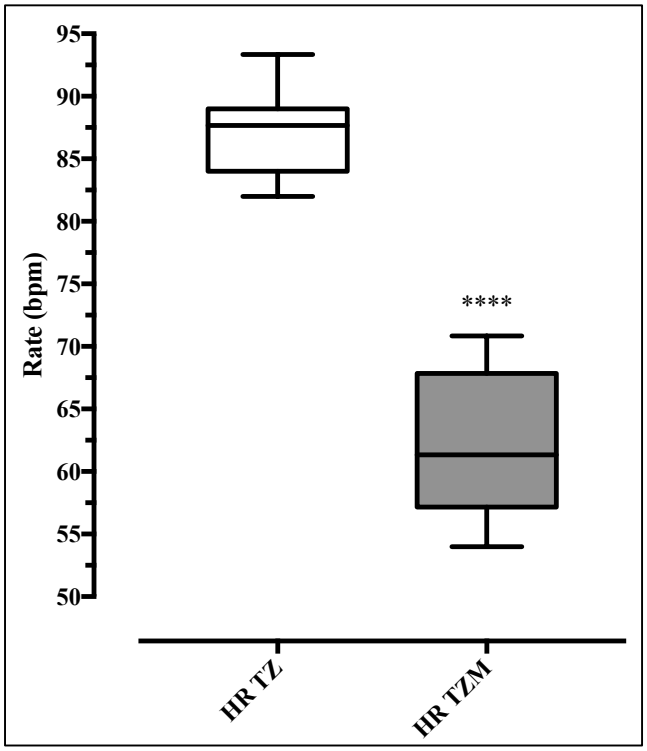


Figure 5.1 Box plot of heart rate (HR) data for both anaesthetic protocols (TZ and TZM). *The boxes and lines represent the interquartile range and median respectively; the whiskers indicate the minimum and maximum. ****: $p \leq 0.0001$*

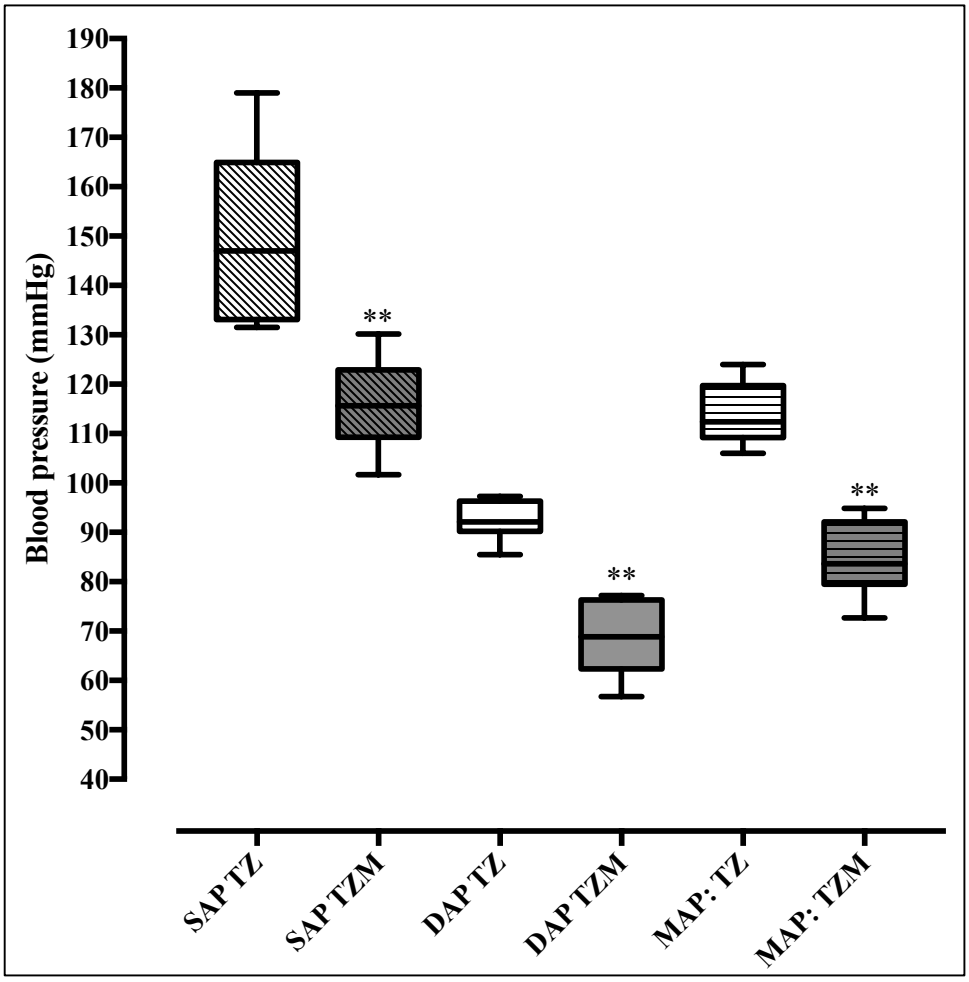


Figure 5.2 Box plot of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure for both anaesthetic protocols (TZ and TZM). *The boxes and lines represent the interquartile range and median respectively; the whiskers indicate the minimum and maximum. **: $p \leq 0.01$*

Mucous membranes were pale-pink to pink and capillary refill times were <2-3 seconds for all animals during both procedures. Arterial oxygen saturation (Figure 5.3) and respiratory rates were lower for the TZM protocol, however the latter of these differences was not found to be statistically significant (Table 5.2).

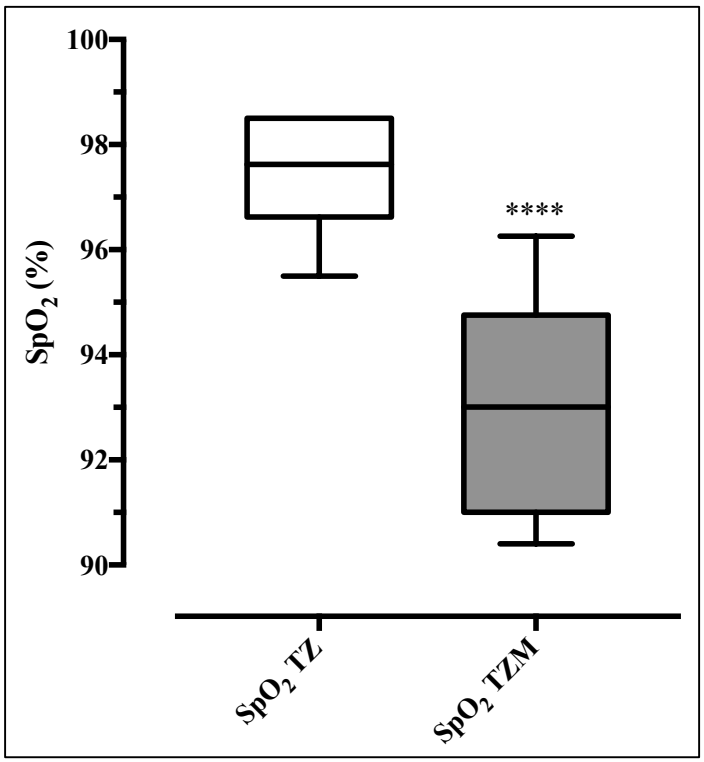


Figure 5.3 Box plot of arterial oxygen saturation (SpO_2) for both anaesthetic protocols (TZ and TZM). *The boxes and lines represent the interquartile range and median respectively; the whiskers indicate the minimum and maximum. ****: $p \leq 0.0001$*

5.3.3 Echocardiography measurements

Data relating to all quantitative echocardiography measurements for both anaesthetic protocols are displayed in Table 5.2. A mild increase in aortic diameter (Ao) and diastolic interventricular septal wall thickness (IVSd) in the TZM compared with the TZ-only anaesthetic protocol were observed. TZM anaesthesia was also associated with a small reduction in: LVIDd, LVIDs, LVPW, EF, LVOT, aortic and pulmonic valve flow velocities, tissue velocity (E' and A'). However, none of these differences were found to be statistically significant. There was no difference in left atrial diameter between the two anaesthetic protocols.

Qualitative (subjective) review by a blinded specialist in veterinary cardiology also confirmed that there was no significant difference in the between the echocardiography images obtained during each of the procedures.

5.3.4 Anaesthesia data

The midazolam oral pre-medication was taken with variable success for procedure 1 (TZ); only two (33%) of the animals took the complete dose offered. A further two animals took 80% and 90% of the dose respectively. The remaining two animals refused the midazolam altogether (Table 5.1).

Pre-anaesthetic aggression and high activity levels were common for both procedures (Appendix 14).

Administration success of the anaesthetic induction agent was 100% in all instances except one (Chimpanzee 2, procedure 1) where the dart did not fully discharge. This animal required the administration of two doses of ketamine before light anaesthesia was achieved (Table 5.1).

There was no significant difference in the time taken to achieve a light plane of anaesthesia (recumbency, muscle relaxation, and an absence of voluntary movements) between the two anaesthetic protocols (Table 5.3). However, the quality of the anaesthetic induction was significantly better (lower score) for the TZM than the TZ-only procedure.

In all cases, the plane of anaesthesia achieved during the TZ-only procedure was scored as being light, whereas for the TZM procedure it was rated as being surgical. The median number of supplemental doses required to achieve/maintain an adequate anaesthetic plane during the TZ procedure was 3.5 (2;7); no additional anaesthetic agents were administered during the second (TZM) procedure. Individual animals' scores are provided in Appendix 14.

Table 5.3 Shows anaesthesia data for all animals and both anaesthetic protocols (TZ and TZM)

Variable	TZ	TZM
Time to light anaesthesia *(min)*	4.5 (1.5;21)	4.3 ± 0.8
Quality of induction	2 (1;4)	1 (1;1)
Degree of muscle relaxation	2.1 ± 0.8	1 (1;1)
Ease of tracheal intubation	2.8 ± 0.8	1 (1;2)*[1]
Combined anaesthetic quality score	7.2 ± 1.9	3 (3;4)*[1]
Plane of anaesthesia	3 (3;3)	4 (4;4)*[1]
Number of supplemental doses required	4 ± 1.8	0 (0;0)*[1]

[1] *: $p \leq 0.05$

5.3.5 Anaesthetic recovery

Five of the six animals' recoveries were rated as being excellent (score 1 out of 4) following both procedures, with no excitation, paddling or involuntary movements being observed. One animal vocalised a lot and was slightly uncoordinated after the TZ procedure and so was assigned a recovery score of 3 out of 4. One of the animals (CH4) also vomited, remained

drowsy and was shivering for a prolonged period following anaesthetic recovery; this prevented her from being mixed back with the rest of the group until the following morning. All recovery scores following the procedure TZM were rated as excellent (score 1 out of 4). Again, however, one animal (CH6) remained sedated for a prolonged period following anaesthetic recovery. Data relating to recovery times are not presented due to the fact that they were intentionally manipulated to allow for animals to be mixed/grouped for transport.

5.4 Discussion

To carry out a full and detailed cardiac assessment including echocardiography in chimpanzees, usually requires that the animals are first immobilised/anaesthetised for reasons of human safety. The effects of the anaesthetic drugs used on the cardiovascular system and therefore also the diagnostic information gleaned are poorly understood.

This prospective blinded clinical cross over study compared the effects of two anaesthetic protocols (tiletamine/zolazepam +/- medetomidine) and evaluated their suitability for the immobilisation of healthy chimpanzees undergoing assessment of cardiac structure and function.

5.4.1 Haemodynamic parameters

TZM anaesthesia was associated was a reduction in heart and pulse rate, and in systolic, diastolic and mean arterial blood pressures when compared with TZ-only anaesthesia. These are well known effects of medetomidine in other species (Dodam et al. 2004; Kallio et al. 1990; Romagnoli et al. 2016; Sabatini et al. 2013; Sinclair 2003). In healthy animals these changes are generally no cause for concern (Lawrence et al. 1996). However, they may not be so well tolerated by the diseased heart, and so these drugs should still be used with caution in animals with diminished cardiac function. That is not to say, however, that TZ-only anaesthesia would automatically be the preferred option when anaesthetising those chimpanzees which are thought to be affected by, or at risk of, cardiovascular disease. The comparatively higher blood pressures which were observed following TZ-only induction reflect an increase in sympathetic drive/tone. This is not only a common feature of dissociative anaesthesia (Maddison et al. 2008; Quesenberry et al. 2004; Tobias & Leder 2011) but might also occur be expected to occur, for

example, due to the comparatively lighter plane of anaesthesia achieved following TZ-only induction reported in this study. Such increased sympathetic drive has the potential to increase myocardial oxygen demand and increase the risk of cardiac arrhythmias. In contrast, alpha-2-agonists have been shown to not only reduce circulating plasma catecholamine levels (Bloor et al. 1992) but also to have a centrally acting anti-arrhythmogenic effect (Hayashi et al. 1991). Given that chimpanzee cardiac deaths have been attributed to terminal arrhythmias (Doane et al. 2006; Sleeper 2009) (rather than heart failure) these factors must not be overlooked when considering the relatively safety of various anaesthetic agents.

5.4.2 Echocardiography parameters

In this study, medetomidine was associated with a reduction in EF, LVOT, aortic and pulmonic valve flow velocities, tissue velocity (E' and A'). Together, these might suggest that medetomidine is associated with a reduction in cardiac output and function, as has been concluded elsewhere (Dodam et al. 2004; Kallio et al. 1990; Napier et al. 2013; Rand et al. 1996; Romagnoli et al. 2016; Sabatini et al. 2013). However, the differences observed in echocardiographic parameter data between the two anaesthetic protocols used in this study were not found to be statistically significant and were similar to the normal ranges published by Sleeper et al. (2014).

The inclusion of medetomidine in the anaesthetic protocol was associated with an increase in diastolic interventricular septal wall thickness (IVSd) and a decrease in left ventricular chamber size (LVIDd/s). These changes can also be seen in pseudohypertrophy, occurring for example due to cardiac tamponade or hypovolaemia (i.e. reduced ventricular filling). It could be hypothesised, therefore, that these changes were falsely induced by as result of lower blood pressure. However, blood pressures were within normal limits published for humans.

Additionally, left ventrivular hypovolaemia (pseudohypertrophy) would also result in an increase in LVPW, whereas the opposite was seen in the chimpanzees. This, and the lack of statistical significance in the differences observed, leads us to conclude that the use of low-dose medetomidine in this study was not associated with a clinically relevant difference in echocardiographic parameters. This contradicts the conclusion drawn by Napier et al. (2013), who compared echocardiographic and blood pressure measurements of seven gorillas during three stages of an anaesthetic protocol. However, these differences can be accounted for by several reasons including variation in: study population (gorillas versus chimpanzees); study design; their inclusion of animals which were receiving treatment for pre-existing cardiovascular disease; the doses of medetomidine (0.02mg/kg versus 0.05-0.07mg/kg) administered, and perhaps above all else; the co-administration of sevoflurane, the potential effects of which cannot not be ignored nor separated from the medetomidine.

5.4.3 Anaesthetic quality

This study described significant differences in the anaesthetic quality, with the addition of medetomidine to the induction protocol resulting in: smoother anaesthetic induction and improved muscle relaxation; easier endotracheal intubation and the achievement of a surgical plane of anaesthesia without the need for supplemental drug administration.

Particularly when working with potentially dangerous animals, anaesthetic plane is not only an important clinical but also safety consideration. The plane of anaesthesia required also depends largely on the intended procedure to be carried out: the requirements of an anaesthetic protocol being utilised to immobilise an animal for movement to another enclosure or for routine health assessment, will differ from those involving nociceptive input such as surgery. This also raises another important issue, which is the anaesthetist/veterinarian's familiarity with the protocol

being used. When anaesthetised with TZ alone, the chimpanzees maintained a degree of muscle tension and seemingly also their reflexes. An individual unfamiliar with TZ-only anaesthesia might mistake this for an indication that the animal is inadequately anaesthetised, and therefore administer unnecessary supplemental doses. Following this study, it was the opinion of the observers that TZ alone at the doses used results in the immobilization of an animal, which is suitable for example: for moving it into another enclosure or hospital facility or; in the hands of a confident/familiar anaesthetist, a non-invasive health assessment. It does not, however, produce a surgical plane of anaesthesia and so if this is required, consideration of the use of further injectable or inhalational anaesthetic agents, or local anaesthesia is necessary.

Considering the anaesthetic quality from the echocardiographers' perspective, adequate muscle relaxation also aids image acquisition and therefore reduces the amount of time required to perform a complete echocardiographic examination. Although data relating to this was not collected nor analysed as part of this study, image acquisition was subjectively reported to be easier during the TZM than the TZ procedure.

5.4.4 Clinical implications

Whilst several great apes housed in North American zoos have been trained for conscious echocardiographic and/or blood pressure assessment (GAHP 2016b), this is not commonplace in European zoos. Additionally, the echocardiographic views that can be obtained even in a well-trained animal are still often of limited diagnostic quality, meaning that these conscious examinations are by no means a substitute for a full detailed echocardiographic study performed under anaesthesia.

The Great Ape Heart Project (GAHP; based at Zoo Atlanta, USA) recommend that great apes of unknown cardiovascular disease status and those undergoing cardiac assessment should not

be anaesthetised using alpha-2-agonists such as medetomidine. This is based on the concern that the effects of these drugs on the cardiovascular system impede meaningful interpretation of echocardiographic assessment and they may also be associated with an increased risk of (peri-) anaesthetic mortality (Brainard 2016; GAHP 2016a). Whilst the findings of the study hereby presented do not contradict this statement, they provide insufficient evidence of such effects to substantiate it. As such, no specific advice relating to the use of specific drugs in the anaesthesia and cardiac assessment of apparently healthy great apes is currently issued routinely by the European vet advisors.

5.4.5 Limitations of the study

The observational and clinical nature of this study meant that there were several variables that could not be controlled. Firstly, induction agents were administered based upon estimated rather than actual bodyweights. However, given that the accuracy of these was very good (mean 5% over/under-estimation), the effect this would have had on the findings is likely minimal.

It was also not possible to control the need for top-ups and therefore the total dose of drugs given: In one instance (Chimpanzee 2, TZ procedure) the dart containing the induction agent did not fully discharge. Observers were therefore required to estimate the actual dose received, and to administer a further two doses of ketamine to achieve light anaesthesia. Due to the dangerous nature of the animals under study, observers were not able to begin all data recording immediately after drug administration; this was especially true for those animals that were in the same enclosure (for example dam and offspring) at the time of induction. There is a possibility that some changes (for example the initial hypertension reported following medetomidine administration observed in other species (Celly et al. 1997; Giovannitti et al. 2015; Kästner 2006; Khan et al. 1999) might therefore have been missed. The time between

administration of the induction agent and the echocardiographic examination being carried out also varied somewhat (Table 5.1). Also, whilst every animal was offered an oral midazolam pre-med prior to anaesthetic induction for the first procedure, it was taken with variable success, and it was not offered at all on the second procedure. In people, oral midazolam is effective in inducing sedation and anxiolysis when used as a pre-medicant for children (McMillan et al. 1992; Sheta & Alsarheed 2009) and adults (Naguib & Samarkandi 2000) undergoing subsequent general anaesthesia. Although cardiovascular effects at low doses are generally minimal, midazolam can also influence heart rate and blood pressure (Nascimento et al. 2007; Dugdale 2011) and so the potential for its use in this study to have impacted upon the findings must not be overlooked.

When discussing relative increases/decreases in any of the parameters measured as part of this study, it must be remembered that the differences are between two different anaesthetic protocols and not in comparison to baseline pre-treatment data. Such comparisons would require these measurements to be taken from awake animals through operant conditioning, therefore deeming this a potential avenue for future research.

The scale used to score recovery incorporated only vocalisation, involuntary/uncoordinated movement and excitation as measures of quality. It did not allow for the capturing of other relevant data, such as shivering, vomiting, and the extent and duration of any persistent sedation. Future studies of this kind, therefore, may be enhanced by the utilisation of a more comprehensive scoring system/scale, ideally designed specifically for the species under study. Ideally, the manipulation of recovery times would also be avoided in futures, to allow for further, more detailed information about the comparative quality of recovery to be gathered.

Additional information and data could have been gathered as part of this study by using additional monitoring techniques (e.g. invasive blood pressure monitoring, blood gases,

pharmacokinetics). Their application, however, would not have been practical, but they nonetheless might provide a useful addition for future work.

An additional and interesting area for further work might also include investigation of the effects of MK-467 on the great ape heart. A peripherally (but not centrally) acting alpha-2-anatagonist, MK-467 has been shown in other species to enhance cardiac function and tissue oxygen delivery but without affecting sedation quality (Pakkanen et al. 2015; de Vries et al. 2016). The potential for its use in both research in this field, and in improving patient safety must therefore be explored.

CHAPTER 6:

A DETAILED STUDY OF GREAT APE CARDIOVASCULAR PATHOLOGY AND SPECIFICALLY IDIOPATHIC MYOCARDIAL FIBROSIS IN CHIMPANZEES

6.1 Introduction

Post-mortem examination of the great ape cardiovascular system has been shown to vary greatly in approach, extent and quality between zoological collections. So too has the quality of information which is provided in the post-mortem reports which are generated. This, along with great variation in the terminology used to describe and diagnose cardiovascular conditions, contributes to an existing lack of understanding about great ape cardiovascular disease, and limits the scope for further research in this field. The first aim of the work reported in this chapter of the thesis was therefore:

1. To utilise a standardised and consistent methodology to identify, describe and characterise the pathological lesions affecting the cardiovascular system of great apes

Specifically, myocardial fibrosis has received considerable coverage within the great ape literature over recent years, and has been associated with sudden death, peri-anaesthetic death, congestive heart failure and cerebrovascular infarction (Chiu & Bodley 2010; Lammey, Lee, et al. 2008; Miller et al. 1999; Munson & Montali 1990; Schulman et al. 1995). In gorillas, this condition, characterised by the presence of myocardial fibrosis for which the cause is unknown, was given the name fibrosing cardiomyopathy (Schulman et al. 1995). There are several issues associated with the use of this term (see Chapter 3), and yet it has been widely accepted by the zoological profession and applied to several pathological lesions in which myocardial fibrosis is present. Myocardial fibrosis, however, is a non-specific response to cardiac insult or injury, of which the causes are many. Its presence and involvement in great ape cardiovascular disease pathogenesis therefore requires further characterisation.

The remaining aims of this chapter of the work reported in this chapter of the thesis were therefore:

2. To analyse and correlate the macroscopic (quantitative and qualitative) and histopathologic features of great ape hearts affected by myocardial fibrosis
3. To compare the identified patterns of pathology with those described in humans and domestic animal species and identify key similarities and differences of interest
4. To use these findings to draw inferences about the possible mechanisms of myocardial fibrosis pathogenesis in great apes

Throughout this chapter, two types of myocardial fibrosis are referred to. Interstitial fibrosis refers to an increase in collagen within the extracellular space. It can be infiltrative, as in amyloidosis and Anderson Fabry's disease, or reactive and stimulated by another pathological process (e.g. in hypertension, diabetes mellitus, idiopathic DCM, HCM, pressure/volume overloading, chronic kidney disease) (Asbun & Villarreal 2006; Heymans et al. 2015; López et al. 2008; Mewton et al. 2011). It can also occur as a normal feature in myocardial ageing. The term replacement fibrosis (also called scarring fibrosis), however, is used specifically to describe the pattern of fibrosis which occurs in response to myocyte damage or loss (see review by Mewton et al. 2011)

6.2 Materials and methods

6.2.1 Tissue and preliminary data collection and processing

Zoological collections and sanctuaries were invited to submit the formalin fixed heart of any great ape that had died in their collection for inclusion in this study. Upon arrival, all formalin fixed heart samples were replaced into fresh 10% neutral buffered formalin in which they were stored until examination, and thereafter. Each sample was assigned a unique study ID.

Prior to heart examination, the following information (where available) was collated and entered an electronic spreadsheet: animal identification (name, number); taxa/species/sub-species; sex; date of birth; date of death; zoological collection at the time of death; date of post-mortem examination and body weight (kg). In some instances (for example, wild caught animals) the exact date of birth was unknown and only a year of birth was provided by the zoo. In these instances, the date of birth was estimated as the 1st of July of that year (as in Chapter 2). Age at death was calculated from the dates of birth and death. Animals were categorised according to their age at death: perinatal, infant, juvenile, sub-adult, adult or aged, using the same categories as defined and used in Chapter 2 of this thesis. Information relating to the animal's clinical history and circumstances of death were summarised and entered as free text. The manner of the death was categorised as follows: euthanasia; anaesthetic-related; due to external causes (for example: attacked by others, accidental death, shot following escape); or natural. Natural deaths were further sub-classified depending on whether they were sudden/unexpected or followed a period of illness. The main post-mortem examination findings were summarised and these and the cause of death added as free text to the spreadsheet. The cause of death was then coded using categories adapted from the ICD-10 classification system already outlined and used in Chapter 2 of this thesis.

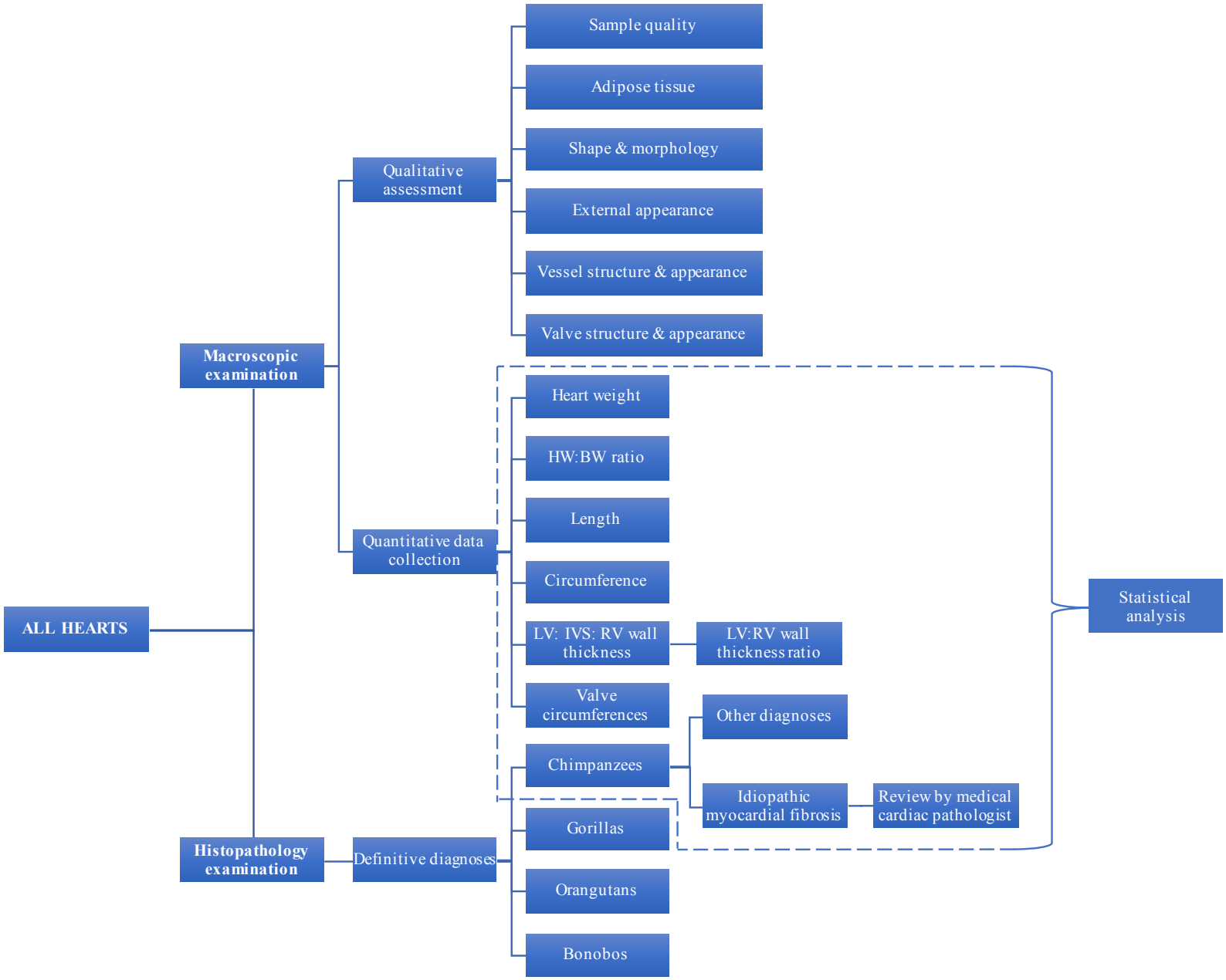


Figure 6.1 Flow diagram showing methodology for macroscopic and histopathological examination of formalin fixed great ape hearts along with steps involved in subsequent data analysis

Figure 6.1 shows the steps involved in macroscopic and histopathologic examination of the hearts, as well as subsequent data collection

6.2.2 Macroscopic examination

6.2.2.1 Qualitative assessment and data collection/processing

Detailed macroscopic examination was carried out according the protocol developed in Chapter 4 of this thesis (Appendix 11). The following were assessed and any remarks or findings documented (as free text) on a purpose designed data collection form (Appendix 12): condition of the sample (extent of any prior sectioning and trimming; quality of tissue preservation; presence of any artefacts associated with fixing, sampling or transport); amount of fat present and its location and; the shape and morphology of the heart and individual chambers. Using criteria described in Table 6.1, the condition of the sample as well as the amount of adipose tissue were scored. Comments were also made on the appearance of the following structures and any lesions or abnormalities described (using free text): epicardium; transverse myocardium; left and right ventricles; left and right atria; aortic, pulmonary, mitral and tricuspid valves; aorta and other major vessels and; the coronary vasculature.

Table 6.1 Table describing scoring system used for condition of heart sample and amount of adipose tissue present

	Score	Description
Condition of sample	***1***	Excellent quality, well preserved sample; has undergone no prior sectioning, trimming or sampling except that described in protocol
	2	Good quality, well preserved sample; perhaps mild artefacts associated with fixing or transport present; has undergone minor prior sectioning, trimming or sampling but this does not impact upon examination nor data collection
	3	Adequate quality sample; minor artefacts associated with suboptimal fixation or transport; has undergone a moderate amount of prior sectioning, trimming or sampling which limits examination and/or data collection slightly
	4	Poor quality sample; inadequately preserved; major artefacts associated with fixing or transport; has undergone significant prior sectioning, trimming or sampling which severely limits examination and data collection
Amount of adipose tissue	***1***	Little to no adipose tissue; less than expected for animal of adequate body condition
	2	Adequate: small to moderate amount of adipose tissue; present within atrioventricular/coronary groove, associated with coronary vessels +/- tip of apex; may extent to cover some (not most/all) of right ventricular +/- left ventricular epicardium; expected amount for animal of adequate/good body condition
	3	Large amount of adipose tissue; protruding from within atrioventricular/coronary groove, associated with coronary vessels and covering significant proportion (most/all) of right ventricular +/- left ventricular epicardium; excessive for animal of adequate body condition (i.e. suggestive of over conditioning)

6.2.2.2 Quantitative data collection

The following quantitative data were also collected: heart weight; heart length (posterior aspect; coronary/atrioventricular groove to tip of apex, Figure 6.2); basal heart circumference (at level of coronary groove; Figure 6.2); left ventricular (LV), right ventricular (RV) and interventricular septal (IVS) wall thickness; aortic (AV), pulmonic (PV), tricuspid (TV) and mitral (MV) valve circumference.

All measurements were taken three times to check for accuracy; if three consecutive identical measurements could not be obtained, a mean of the three measurements was calculated and used.

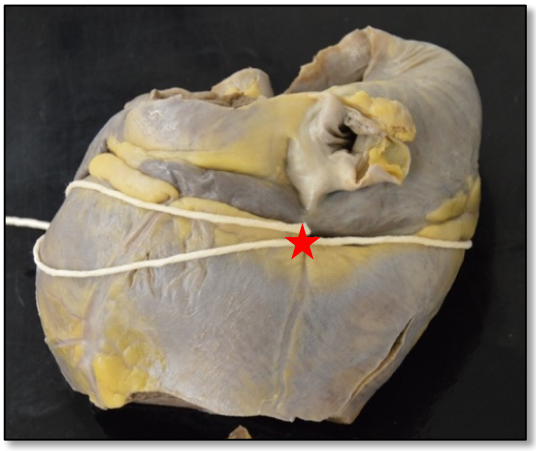


Figure 6.2 Demonstrates the measuring of heart circumference at the coronary groove using string and shows the point (red star) from which the heart length is measured

6.2.3 Tissue sampling for histopathology

Where sample condition permitted, a minimum of twelve tissue samples were taken from pre-determined and consistent locations and placed into cassettes. These included myocardium from the: anterior, posterior and lateral left and right ventricular walls; anterior and posterior portions of the interventricular septal wall and right ventricular outflow tract. Samples of the aorta, sino-atrial nodal region and atrioventricular nodal regions were also taken. Additional tissue samples of anomalies or lesions detected as part of the examination were also taken as required. All samples were then processed, sectioned and stained (with haematoxylin and eosin stain) for histopathological examination.

6.2.4 Histopathological examination and data collection

Each individual slide was then examined under the supervision of a specialist pathologist who is a member of the European College of Veterinary Pathology, and qualitative descriptions relating to the findings were added to the data collection form. Histological diagnoses were generated and interpreted alongside the findings of the macroscopic examination. These were combined into final reports, which were issued to the zoological collection submitting the sample.

A selection of the cases were also reviewed by an expert in (human) medical cardiovascular pathology and sudden cardiac death. The histopathological features were compared with those cardiac conditions which have been well documented and described among people.

6.2.5 Data analysis

Statistical analyses on quantitative data were carried out using Microsoft Excel (Microsoft 2010) and GraphPad Prism 7 for MacOS X (2016). Data were assessed for normality using a Shapiro-Wilk test. Quantitative data were compared with normal reference ranges published for use in humans (Kitzman et al. 1988; Kitzman & Edwards 1990; Lucas 2011; Sheppard 2012; Sheppard & Davies 1998) and were grouped by sex, cause of death, and by the presence/absence of pathological lesions. Parametric data were compared using unequal variance (Welch) t-tests; the Mann-Whitney test was used for non-parametric data. Confidence intervals were set at 95% and p-values of <0.05 were considered significant. Normally distributed data are expressed as mean and standard deviation, and non-normally distributed data as median, range (min; max) unless otherwise stated.

6.3 Results

6.3.1 Study population

Demographic information relating to the population of animals under study is provided in Table 6.2. A total of 34 (5 bonobo; 22 chimpanzee; 5 gorilla; 2 orangutan) hearts were examined. Almost two thirds (n=22/34; 65%) of the animals were male; the remaining 35% (n=12/34) being female. Mean age at death for the animals under study was 27.94 (± 15.02) years. The hearts were taken from two infant, two juvenile, six sub-adult, fourteen adult and ten elederly/aged animals. More than one quarter (n=9/34; 26%) of the deaths were due to disease of the circulatory system; 18% (n=6/34) were due to respiratory disease; 18% (n=6/34) due to external causes; 15% (n=5/34) due to diseases of the digestive system; and 9% (n=3/34) due to infectious disease. The cause of death was categorised as miscellaneous/other for one animal under study, and was unknown for four.

Table 6.2 Demographic and cause of death information relating to the study population (n=34)
Abbreviations: M: male; F: female. AD: adult; AG: aged/elderly; IN: infant; JU: juvenile; SA: sub-adult. CI: circulatory; DI: digestive; EX: external causes; IN: infectious; MI: miscellaneous/other; RE: respiratory; UK: unknown

	Study ID	**Sex**	**Age at death** *(yrs)*	**Age category at death**	**Bodyweight** *(kg)*	**Cause of death category**
Bonobos	**B1**	M	11.49	SA	31.3	RE
	B2	F	1.64	JU	3.8	RE
	B3	F	0.45	IN	2.2	RE
	B4	M	34.92	AD	46.0	DI
	B5	M	36.01	AG	41.7	EX
Chimpanzees	**C1**	M	9.98	JU	57.8	EX
	C2	M	37.40	AG	72.0	CI
	C3	M	33.45	AD	59.0	CI
	C4	M	33.25	AD	61.0	CI
	C5	M	28.00	AD	69.4	CI
	C6	M	31.00	AD	45.0	CI
	C7	M	21.87	AD	66.7	CI
	C8	M	39.42	AG	69.5	EX
	C9	F	32.02	AD	48.0	EX
	C10	F	24.83	AD	40.3	RE
	C11	F	42.05	AG	47.0	IN
	C12	M	11.37	SA	45.0	UK
	C13	M	13.41	SA	50.0	UK
	C14	M	19.93	SA	66.0	UK
	C15	F	46.32	AG	44.0	IN
	C16	M	52.37	AG	50.0	UK
	C17	M	45.93	AG	58.0	CI
	C18	M	18.90	SA	60.5	CI
	C19	F	25.25	AD	51.0	CI
	C20	M	21.96	AD	59.0	RE
	C21	F	58.76	AG	47.2	MI
	C22	M	21.63	AD	50.0	DI
Gorillas	**G1**	M	27.89	AD	190.0	RE
	G2	F	51.78	AG	82.0	DI
	G3	F	43.57	AG	65.0	DI
	G4	M	13.60	SA	114.0	IN
	G5	F	32.69	AD	72.5	DI
Orangutans	**O1**	F	0.16	IN	2.2	EX
	O2	M	26.60	AD	120.0	EX

6.3.2 Macroscopic examination: qualitative data analysis

6.3.2.1 Sample quality

Sample quality was overall very good: 68% (n=23/34) of the hearts were given a score of one (excellent; see example Figure 6.3) or two (good), meaning that the samples were well preserved with only minimal artefacts (if any) being present, and that only minor prior sectioning or trimming had taken place. Sample quality was scored as being adequate (n=8) or poor (n=3) for the remaining hearts, meaning that examination and subsequent data collection was somewhat limited and therefore incomplete for 32% of the hearts examined.

6.3.2.2 Adipose tissue

Data relating to the amount of adipose tissue associated with the heart was not obtainable for three animals due to the condition of the sample or extent of prior sectioning which it had undergone. Of the remaining (n=31) hearts, the majority (n=25/31; 81%) had an adequate amount of adipose tissue present, i.e. a small to moderate amount within the atrioventricular/coronary groove, associated with the coronary vessels and tip of the apex (see example, Figure 6.3). In some cases, the adipose tissue also extended to cover some (but not all) of the right +/- left ventricular epicardium. Two (n=2/31; 6%) of the animals had little to no adipose tissue associated with them: an adult male chimpanzee with a history of chronic colitis which had caused him to become cachexic/emaciated and a sub-adult male chimpanzee which was subsequently diagnosed with left ventricular hypertrophy (of unknown cause). Four (n=4/31; 13%) of the animals had a large (excessive) amount of adipose tissue associated with their hearts: an elderly male chimpanzee, an elderly female chimpanzee and two elderly female gorillas.

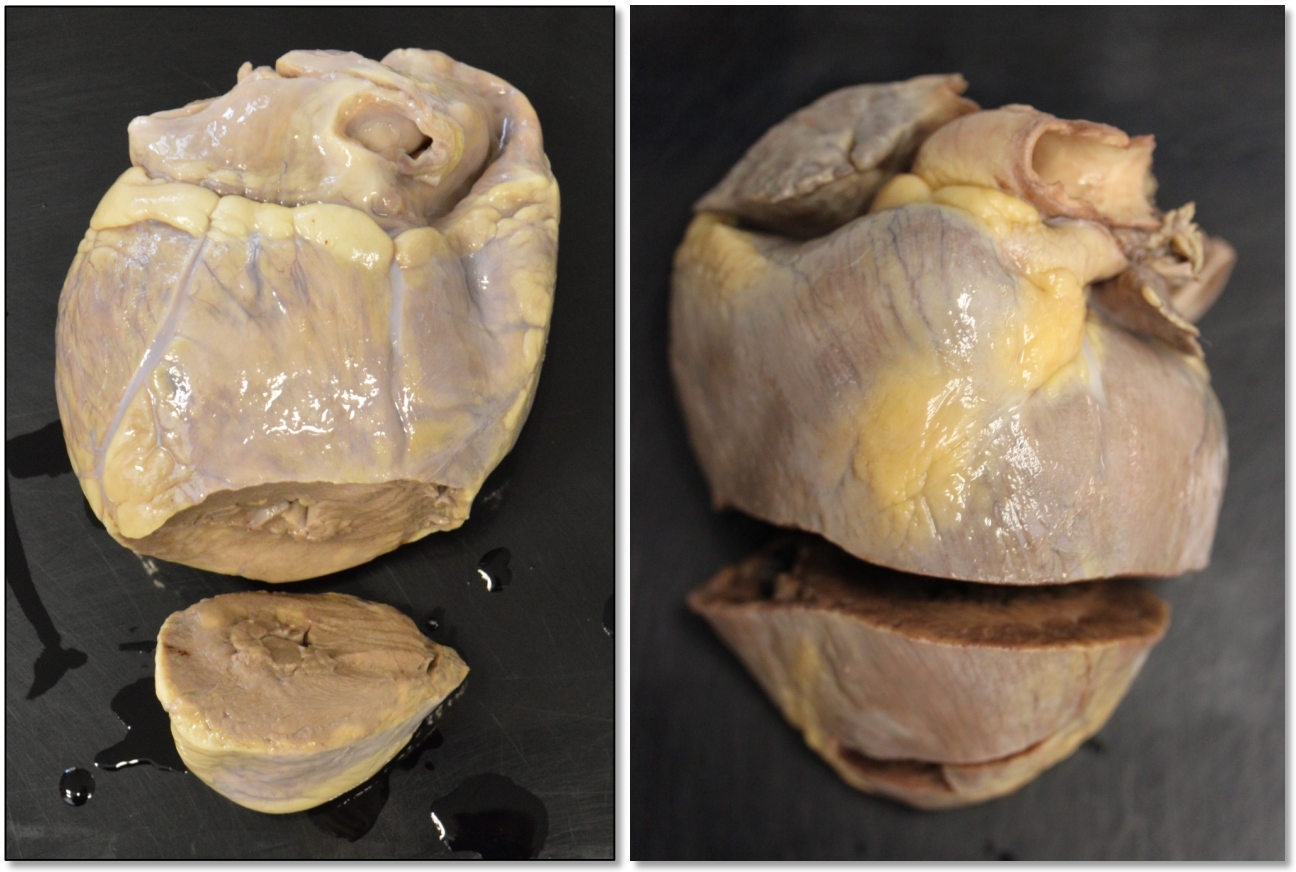


Figure 6.3 Images showing the typical appearance of an excellent quality (score 1 out of 4), well preserved, formalin fixed, chimpanzee heart, with an adequate (score 2 out of 3) amount of adipose tissue present

The images show the posterior (left) and anterior (right) aspects of the heart. The heart has been transected across the lower third of the apex, approximately 3cm from the apical tip, as per the examination protocol (Appendix 10). Only a small (<1cm) piece of apical myocardium is missing (see image, right); this had been sampled and sections frozen at -80°C and in RNA-later as per the protocol. Note the presence of adipose tissue (pale, yellowish) within the coronary groove, associated with the coronary vessels, right ventricular free wall and tip of the apex.

6.3.2.3 Shape and morphology

Data relating to the shape of the heart was not obtainable for three animals due to the condition of the sample or extent of prior sectioning which it had undergone. The majority (n=19/31; 61%) of the remaining hearts were normal in shape. The subjective impression of left ventricular/septal hypertrophy was noted in six animals. However, only one of these was found to have evidence of true hypertrophy on histological examination. Similarly, the subjective impression of dilation, bulging or rounding of one or more areas of the heart was reported in 6 animals. However, only one of these animals was diagnosed with a cardiac condition (ARVC) which would cause such dilation. This therefore demonstrates the importance of not carrying out subjective assessment of heart size and structure alone, and not over interpreting macroscopic findings.

The foramen ovale was found to be patent in the two-month-old orangutan heart examined (incidental finding). No congenital abnormalities were identified in any of the hearts examined.

6.3.2.4 External appearance

Changes to the appearance of the epicardium were common, with only 21% (n=7/34) of the hearts exhibiting no changes. The remaining hearts all showed evidence of mottling or discolouration. These areas of discolouration were, in most cases, reported to be pale or whitish in colour, and in some instances were associated with indentation of the epicardial surface (more appreciable on transverse examination, see Figure 6.5). In one case, the epicardium was reported to be reddish, as opposed to pale, in colour. The changes ranged from being mild and/or focal, to being severe and multifocal or diffuse. Occasionally, small (1-2mm) focal dark red-brown circular lesions were identified on the epicardial surface, mostly associated with the

epicardial fat or surrounding the coronary vessels; these were assumed to be agonal haemorrhages and interpreted as an incidental finding. Examples of a normal versus a severely mottled heart are shown in Figures 6.4a & b.

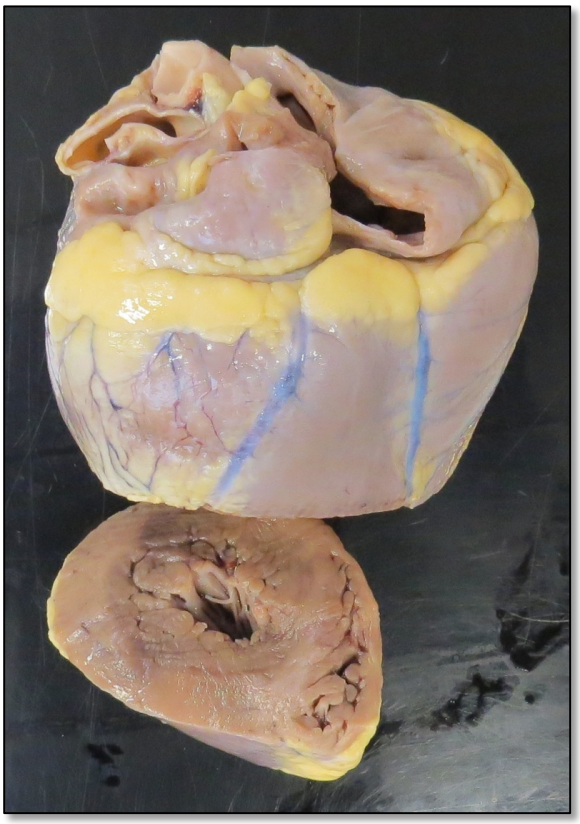


Figure 6.4a (left) Image showing appearance of a normal chimpanzee (C12) heart, with no epicardial mottling

Note the smooth, uniform appearance of the epicardium in comparison with that shown in Figure 6.3b (below)

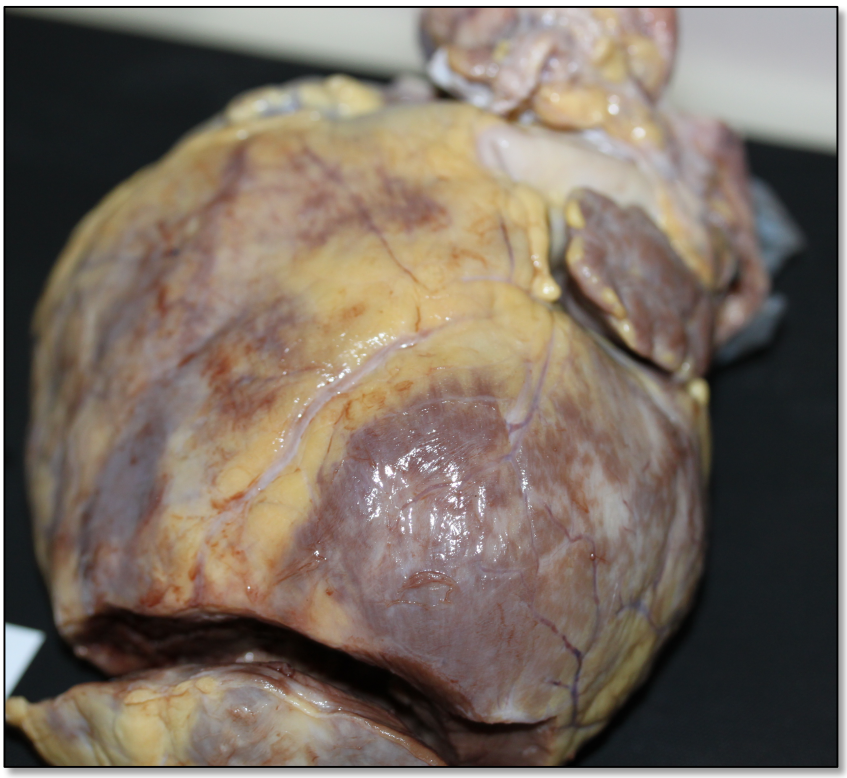


Figure 6.4b (right) Image showing appearance of chimpanzee (C4) heart, with marked epicardial mottling

Note the heterogenous appearance of the epicardium; the multi-focal coalescing pale areas were associated with indentation of the myocardium (see Figure 6.5)

6.3.2.5 Transverse myocardium appearance

On transverse examination of the myocardium, the indentations of the epicardium which, in some animals were present and corresponded with areas of epicardial pallor, can be better appreciated (Figure 6.5).

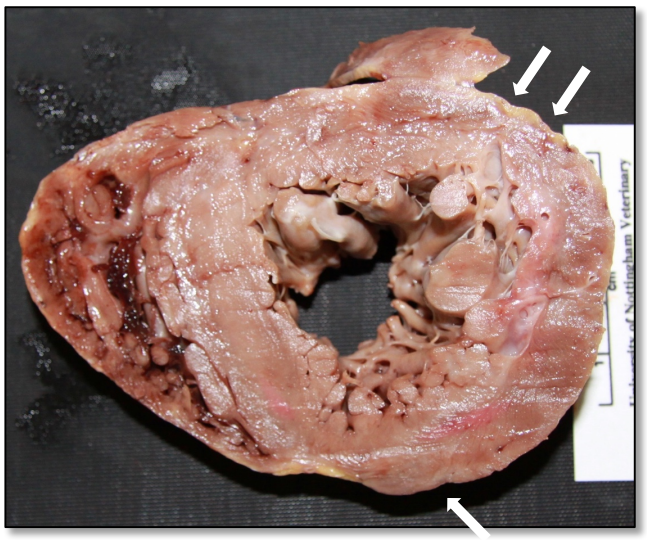


Figure 6.5 Image showing appearance of chimpanzee (C4) heart, with indentation of the myocardium
These indentations (white arrows) were associated with the multi-focal coalescing pale areas on the epicardium (shown in Figure 6.3b)

Pallor or mottling of the transversally sectioned myocardium was also common, being reported in 65% (n=22/34) of hearts examined. In some instances, it was only the sub-epicardial or sub-endocardial myocardium and papillary muscles that were pale in appearance (Figure 6.6). These areas were not associated with any histological lesions and were therefore interpreted to have been caused by variation in formalin uptake and subsequent fixation.

In others, the pale areas were more circular or streak-like in appearance and were randomly distributed throughout the myocardium. These areas were more often associated with genuine pathological change, such as myocardial fibrosis (see later).

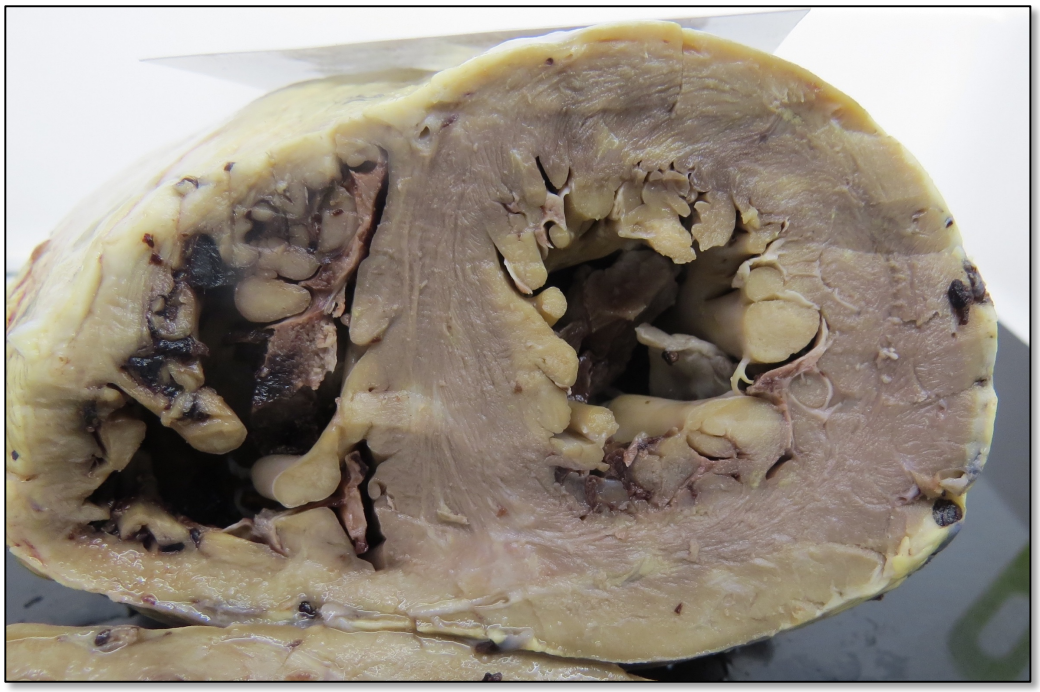


Figure 6.6 Image showing fixing artefact affecting the myocardium of a chimpanzee

Note the difference in appearance of the pale sub-endocardial and sub-epicardial myocardium compared with the darker, pink-brown mid-myocardium

In some of the hearts examined, the mid-myocardium was found to be pink in colour (Figure 6.7). This again was not associated with any pathological lesions and was interpreted as being due to inadequate formalin penetration and therefore a fixing artefact.

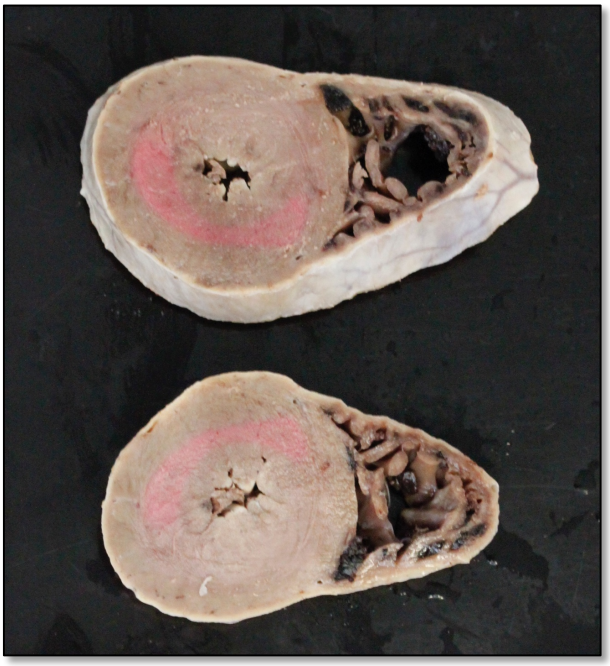


Figure 6.7 Image showing fixing artefact affecting the transverse myocardium of a bonobo

Note the pink colouration of the mid-myocardial tissue compared with the darker, pale brown (normal) tissue which surrounds it.

Also note the impression of apparent left ventricular hypertrophy in this heart. However, there was no significant increase in heart weight or left ventricular wall thickness, and no hypertrophy was diagnosed following histopathological examination.

6.3.2.6 Vessel structure and appearance

Macroscopic lesions associated with the vessels were uncommon; changes were reported in only 21% (n=7/34) of the hearts examined. Focal areas of aortic endothelial discolouration (pale or whitish in five; yellow or orange in two), which were sometimes raised or thickened, were identified in seven animals. In some instances, these lesions were slightly firmer than the surrounding tissue on palpation, and in two cases they were found to be gritty in texture. No abnormalities of the coronary vasculature were identified in any of the hearts following macroscopic examination.

6.3.2.7 Valve structure and appearance

Lesions of the heart valves were uncommon, with >76% appearing macroscopically normal. Changes identified included: firm nodular thickening (endocardiosis, Figure 6.8) of the mitral (n=2), tricuspid (n=1) and pulmonic (n=1) valves); and non-nodular thickening/stiffening of the aortic valve (n=2).

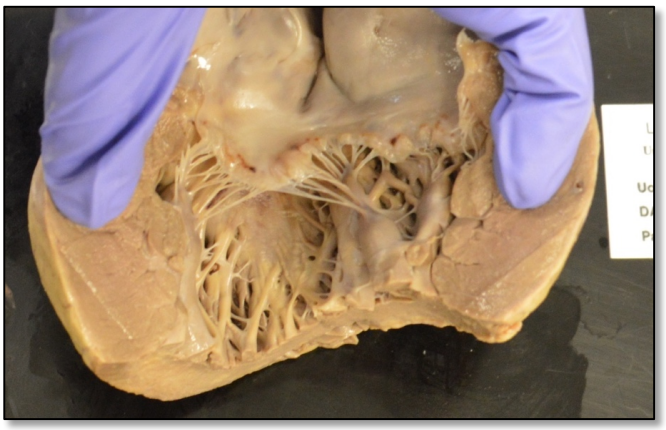


Figure 6.8 Image showing endocardiosis of the mitral valve in an elderly female chimpanzee

Note the multiple nodular masses present along the valve margin

Brown mottling and haemorrhage associated with the endothelium of the mitral valve was also reported in one animal, but was interpreted an incidental finding.

The case of tricuspid valve endocardiosis was also associated with shortening/contraction of the chordae tendineae; this animal had been diagnosed with a cardiac murmur and tricuspid regurgitation ante-mortem. This was, however not considered to be clinically significant, nor the cause of this animal's death.

6.3.3 Histopathology examination: definitive (final) diagnoses

Following histologic examination, the following diagnoses were made.

6.3.3.1 Bonobos

No evidence of cardiovascular lesions were detected in two (n=2/5; 40%) of the bonobo hearts examined. Both animals (B2 & B3) were aged <2 years at the time of death, both of which were due to respiratory infection.

One animal, a sub-adult (11-year-old) male bonobo (B1) died following a period of illness due to a bacterial meningitis which occurred as a sequel to a widespread co-infection with respiratory syncytial virus (RSV) and *Streptococcus pneumoniae*. Despite this animal's young age and non-cardiac cause of death he was found to have focally extensive replacement fibrosis affecting the left ventricular papillary muscle. He also had scattered acute to subacute cardiomyofibre necrosis with a mixed cellular infiltrate. The acute necroses were likely associated with death (agonal, incidental) and the subacute necroses interpreted as being related to the ongoing systemic bacterial infection and likely terminal sepsis.

Another animal, an elderly male bonobo (B4), was diagnosed with arrhythmogenic right ventricular cardiomyopathy (ARVC): multifocally within the myocardium, vast areas of cardiomyocytes had been replaced by expansive fatty to fibrofatty (+/- granulation) tissue. These changes were mild to moderate and were most prominent within the right ventricle, the right side of the interventricular septum and especially the right ventricular outflow tract. Associated with these areas were multifocal, random scattered infiltrates of lymphocytes. Multifocally, within the left ventricle, areas of replacement fibrosis were also identified. Also within the left ventricle were occasional lytic or fragmented cardiomyofibres, which were accompanied by a mild neutrophilic infiltrate. This animal had been diagnosed with a

cardiomyopathy ante-mortem, for which he was receiving ACE inhibitor therapy and which had resulted in a clinical improvement. Three days prior to his death he had been attacked by the females in the group and had since been housed separately to be treated for a suspected gastric ulcer. His death was reported by the zoological collection and pathologist to be due to a perforated gastric ulcer and subsequent peritonitis.

Finally, B5 was another elderly male bonobo who was euthanased after sustaining non-reparable injuries inflicted by his cage-mates. Detailed examination of his heart revealed multifocal, minimal to mild myocardial replacement fibrosis and small, multifocal lymphocytic cell clusters. These changes, however were very mild, and were not interpreted to be associated with his death.

6.3.3.2 Gorillas

No pathological lesions were identified in two (G2 & G3) of the five (40%) gorilla hearts examined. An additional gorilla (G5) heart was found to be affected by minimal to mild multifocal, randomly distributed, perivascular and replacement fibrosis affecting the left ventricle, and minimal diffuse interstitial fibrosis within the interventricular septum. However, given that these changes were very mild and affected only small areas of the tissues examined, they were interpreted as being of no clinical significance to this animal. All three of these cases were adult or elderly females which died as the result of disease of the digestive system.

G1 was an adult male gorilla which died during anaesthesia for the investigation of a suspected dental abscess. The submitting zoological collection and pathologist cited that his death was caused by upper airway obstruction due to a peri-pharyngeal abscess and glossitis. Nonetheless, this animal was found to have multifocal mild to focally extensive replacement fibrosis present

throughout the left and right ventricular and interventricular septal myocardium, as well as multifocal, minimal to mild interstitial and perivascular fibrosis. Numerous small to medium sized arteries were also found to be affected by thickening of the tunica media, suggesting a possible underlying hypertensive aetiology for the fibrosis in this animal (Figure 6.9). The degree to which this cardiac disease contributed to the death of this animal was uncertain.

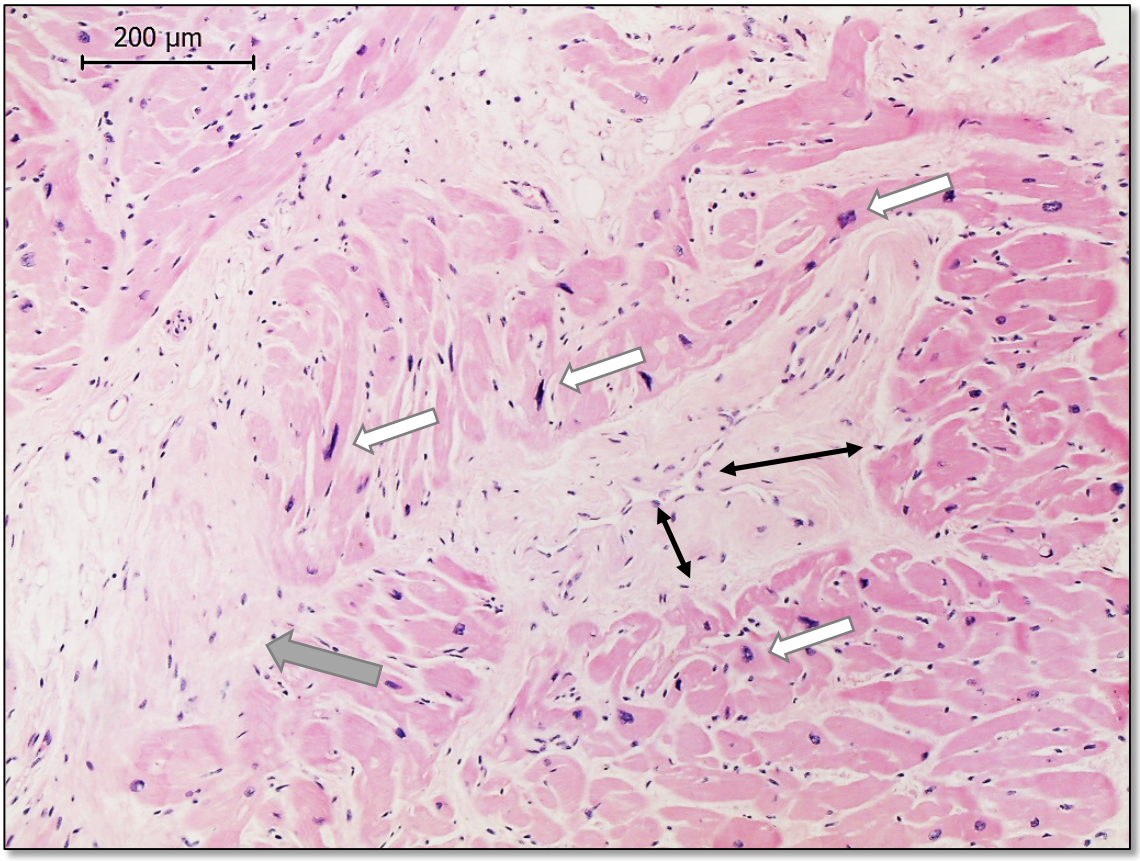


Figure 6.9 Image showing tunica media thickening and associated reduction in lumen size of an arteriole (black double headed arrows) in an H&E stained right ventricular myocardial section of a gorilla (G1) heart.

Adjacent to this is an area of replacement fibrosis (grey arrow). Nearby myocytes also demonstrate reactive anisokarysosis and anisocytosis (white arrows).

G4 was a sub-adult (13-year-old) male gorilla which was euthanased due to chronic hepatic echinococcosis. Despite his young age and non-cardiac cause of death, examination of his heart revealed minimal to focally moderate replacement fibrosis with associated mixed leukocytic infiltration affecting the left ventricular and interventricular septal myocardium; acute to per-acute multifocal myocardial necrosis and contraction bands with associated mixed leukocytic infiltration affecting the left ventricle; minimal diffuse interstitial fibrosis affecting the left and right ventricular and interventricular septal myocardium and; a focal fibrotic plaque in the aorta.

6.3.3.3 Orangutans

No cardiovascular lesions were detected in either of the two orangutan hearts examined. One of these was a case of infanticide (trauma inflicted by adult male on an infant less than two months of age); the other an animal male which had to be shot after he escaped from his enclosure and could not be contained.

6.3.3.4 Chimpanzees

Chimpanzees were the taxa from which the greatest number of samples (n=22) were examined. The prevalence of cardiovascular disease was found to be very high, with only three (n=3/22; <14%) chimpanzees being found not to have evidence of significant cardiovascular disease. Two of these were sub-adult (estimated 11 & 13 years old) male, wild-born chimpanzees (C12 & C13) from an African sanctuary. For both animals, the cause of death was unknown but suspected to be infectious or toxic. The remaining animal was an adult (20-year-old) male zoo-housed chimpanzee, which died during anaesthetic recovery due to air sacculitis and secondary

sepsis. Within the left ventricular myocardium there were a few, scattered, very small areas of mild replacement fibrosis as well as scattered small lymphoplasmacytic infiltrates. However, these were not associated with significant myocardial inflammation nor damage, and were interpreted as being of no clinical significance to this animal and not associated with his death (incidental finding).

C6 was an adult male chimpanzee that had undergone numerous prior anaesthetic procedures for the surgical treatment of a herniated intervertebral disc. He was reported by the zoological collection to have never fully recovered from the most recent anaesthetic, 3 days prior, and to have died due to myocardial infarction. Detailed examination of his heart confirmed the presence of a left ventricular myocardial infarction as well as mild, scattered dystrophic mineralization of the myocardium of unknown cause and significance. However, this animal did not show any other signs of an underlying cardiovascular condition.

C10 was an adult female chimpanzee which had a history of multiple health problems since arrival at the submitting zoological collection. She underwent general anaesthesia to investigate her ill health and at this time, numerous pathological lesions including a large thrombus in her left ventricle were detected. She was treated but died overnight, the cause being identified as a chronic infection of the respiratory tract, liver, spleen and other organs. Cardiac examination findings were supportive of this, as she was diagnosed with: acute to subacute fibrinous multifocal endocarditis, epicarditis and pericarditis with multifocal, randomly distributed acute to chronic vascular thrombi within numerous sections examined and scattered acute myofibre degeneration and necrosis with associated neutrophilic infiltration affecting the left ventricle. These changes are consistent with a diagnosis of a systemic septic process, which was having secondary inflammatory and ischemic effects on the heart. However, this animal also showed evidence of an underlying cardiovascular condition which was similar in nature to those

described in Table 6.3. The heart also appeared macroscopically enlarged (hypertrophy) and histologically there was moderate diffuse interstitial and perivascular fibrosis with multifocal thickened cardiomyofibres and enlarged nuclei.

C11 was an elderly (42-year-old) female chimpanzee which was euthanased as a result of being hepatitis B positive and therefore not being able to be moved to another collection. The lesions observed in this animal were similar to those outlined in Table 6.3 (see later) in that it was characterised by a minimal to mild chronic, multifocal replacement fibrosis with a mild lymphocytic infiltration. This animal, however, exhibited a mild thickening of the tunica media in numerous small to medium sized arteries, suggestive of a potential underlying hypertensive aetiology or involvement. In addition, unlike in those animals in Table 6.3, the areas of replacement fibrosis were not random: they instead appeared to show a predilection for the perivascular and sub-epicardial myocardium.

C14 was a sub-adult (20-year-old) male chimpanzee which died under anaesthesia. The animal was believed to otherwise be healthy; the only abnormality reported by the zoological collection being that he had recently been very nervous following the transfer of another male animal out of the group, and had been plucking his forearms. Post-mortem examination of all other organs was reported to be unremarkable. Cardiac examination revealed: chronic, mild, random, multifocal myocardial interstitial fibrosis; mild, mixed cellular infiltration and; occasional multifocal, random, single cell cardiac myocyte necroses with accompanying mixed cellular infiltration. Replacement fibrosis was not a feature. Since these changes were mild they were interpreted as being incidental and unlikely to explain the unexpected death of this animal. There was also the impression of multifocal, mild tunica media thickening and mild perivascular fibrosis, which might be suggestive of hypertension.

C17 was an elderly (>45-year-old) male chimpanzee with a history of diabetes mellitus. The presence of a severe hydrothorax and liver enlargement were detected on health check examination after he had become dyspnoeic and anorexic, and he was subsequently euthanised. Detailed cardiac examination revealed an acute dissecting aneurysm of a branch of the coronary artery within the left ventricle. Histologically, multifocal, acute single cell necroses, associated neutrophilic infiltration and chronic replacement fibrosis of the sub-endocardial left ventricular and papillary myocardium were also identified; these changes are likely associated with the dissecting aneurysm causing cardiac muscle hypoxia and insufficiency. This animal also had a focal intimal aortic fibrofatty plaque identified which was interpreted as an incidental finding.

C18 was a sub-adult (18-year-old) male chimpanzee with a history of recurrent air sacculitis for which he had undergone recent surgery and was receiving medical therapy. However, despite treatment he died 3 weeks after initiating this treatment. According to the post-mortem report provided by the zoo, his cause of death was cardiac failure due to myocardial fibrosis. However, more detailed examination of the heart revealed cardiomegaly and gross hypertrophy. This was further substantiated by the histopathological finding of multifocal to diffuse cardiomyofibre hypertrophy with multifocal disarray; severe, multifocal to coalescing, randomly distributed, subacute to chronic replacement fibrosis with moderate lymphocytic infiltrates affecting the left ventricle and left ventricular portion of the interventricular septum and; multifocal acute single cell necroses and mineralization. From the examination conducted, it was not possible to determine whether the left ventricular hypertrophy in this animal was physiological and secondary for example, to systemic hypertension, or primary due to hypertrophic cardiomyopathy.

C19 was an adult (25-year-old) female which was reported to have died suddenly with no prior clinical signs. Detailed histological examination identified multifocal to focally extensive proliferations of lymphocytes and lymphoblasts affecting all cardiac structures (endocardium, myocardium and epicardium), which was later confirmed to be lymphoma. Also identified was a chronic moderate, diffuse, interstitial fibrosis as well as multifocal focally extensive replacement fibrosis. This was most prominent on the left side of the heart and was accompanied by prominent myocyte hypertrophy and disarray, suggesting a possible diagnosis of left ventricular hypertrophy.

The histopathological findings for all other (n=12) chimpanzee hearts examined were similar, and were all characterized by the presence of replacement and interstitial fibrosis. Unlike all of those described so far, no possible underlying cause for the fibrosis could be identified in any of the sections examined. The main histological features of this condition (referred to idiopathic myocardial fibrosis) are displayed in Table 6.3 and are hereby described in greater detail.

6.3.4 Chimpanzee idiopathic myocardial fibrosis (IMF)

The areas of replacement fibrosis typically consisted of mature fibrous tissue, which may or may not have contained a central region (core) of adipose tissue (Figures 6.9a&b, 6.10a). In some instances, active fibrous tissue formation (granulation tissue; indicated by the presence of many fibroblasts and neovascularisation; Figure 6.11) was evident; in these cases, the replacement fibrosis was described as being chronic-active. The fibrotic lesions themselves were randomly distributed showing no obvious predilection for the sub-endocardial, mid-myocardial nor sub-epicardial myocardium.

In most instances, the surrounding myocardium exhibited reactive change typified by anisocytosis, anisokaryosis +/- myofibre disarray (Figure 6.10a). These changes, however, were mild and restricted only to the perilesional myocardium (i.e. they were not more generalised as in left ventricular hypertrophy or hypertrophic cardiomyopathy).

Mild cellular infiltration was a common accompaniment to these fibrotic lesions; infiltrates were usually predominated by lymphocytes, but also contained prominent numbers of histiocytes, lesser neutrophils and plasma cells, and frequently small numbers of eosinophils (Figure 6.10b). This cellular infiltrate was generally restricted only to the fibrotic lesions; i.e. it was not associated with a more generalized or diffuse inflammatory change, as would be seen for example in cases of inflammatory myocarditis. The extent of cellular infiltration varied between hearts/animals, and ranged from minimal to moderate. In some animals, occasional scattered small lymphocytic interstitial or perivascular infiltrates were also present, although these were also observed in other animals with no other cardiovascular lesions and are known in humans to be a normal finding (Sheppard, personal communication). They were therefore interpreted as being incidental.

There was an increase in connective tissue within the interstitial (inter-myofibre) space in all the hearts examined. This ranged in severity from minimal to focally marked, and was typically multifocal or diffuse in distribution.

Multifocal acute myocardial necroses +/- associated mixed cellular infiltrates were also a feature in nine (n=/12; 75%) of these chimpanzees. The very acute changes like contraction bands (transverse bands visible in myofibres, associated with myocyte hypercontraction), fragmentation, and single cell necroses can occur due to terminal hypoxia and were therefore often interpreted as agonal changes related to terminal dysrhythmic events. Other changes of

acute myocardial degeneration such as fragmentation, vacuolation and thin wavy fibres were also observed in five (n=5/12; 42%) of the hearts examined.

The left ventricle and interventricular septal myocardium were most preferentially involved, although changes were often evident throughout all chambers.

Of those chimpanzees affected by idiopathic myocardial fibrosis, 75% (n=9/12) were male. Mean age at death was 34.54 (± 13.68) years. The cardiovascular condition identified was the cause of death in 42% (n=5/12) of the animals; their deaths were sudden (n=3) or peri-anaesthetic (n=2).

6.3.4.1 Comparison with human cardiomyopathies

The pathological lesions identified were reviewed by an expert in (human) medical cardiovascular pathology and sudden cardiac death and compared with those which have been well documented and described among people. It was confirmed that these conditions did not resemble, for example, dilated (DCM), hypertrophic (HCM), arrhythmogenic right ventricular (ARVC), restrictive cardiomyopathy (RCM) or any other of the more 'typical' cardiomyopathies. The opinion was that changes in the examined chimp hearts were most comparable to idiopathic myocardial fibrosis in humans. The main exception, however, was that the degree of cellular infiltration was marginally higher than would be expected in similar cases in humans.

Table 6.3 Details relating to the main histopathological features of idiopathic myocardial fibrosis observed in twelve chimpanzees

Cause of death categories include: CI, circulatory; DI, digestive system disorders; EX, external causes; IN, infectious; MI, miscellaneous/other; UK, unknown

Study ID	Sex	Age at death *(yrs)*	COD	Manner of death	Replacement fibrosis	Myofibre hypertrophy	Myofibre disarray	Myocardial degeneration	Cellular infiltration	Myocardial necrosis	Interstitial fibrosis
C1	M	9.98	EX	External	Chronic-active Mild Multifocal Random Mature fibrous and granulation tissue	None	Mild Perilesional	None	Minimal to mild Lesion associated Mixed	Acute Multifocal Random	Minimal to mild Diffuse
C2	M	37.40	CI	Peri-anaesthetic	Chronic-active Moderate to marked Multifocal Random Mature fibrous, fibrofatty and granulation tissue	Mild Perilesional	Mild Perilesional	Multifocal Random	Mild to focally moderate Lesion associated Mixed	Acute Multifocal Random	Moderate to marked Multifocal
C3	M	33.45	CI	Peri-anaesthetic	Chronic-active Mild to focally severe Multifocal Random Mature fibrous, fibrofatty and granulation tissue	Mild Perilesional	Mild Perilesional	Multifocal Random	Minimal to mild Lesion associated Mixed	Acute Multifocal Random	Moderate Diffuse
C4	M	33.25	CI	Sudden	Chronic Moderate to focally marked Multifocal Random Mature fibrous and fibrofatty tissue ***(Fig. 6.9a&b)***	Mild Perilesional ***(Fig. 6.10a)***	Mild Perilesional	Multifocal Perilesional ***(Fig 6.11)***	Mild Lesion associated Mixed ***(Figure 6.10b)***	Acute Scattered, multifocal Random	Mild Diffuse ***(Fig. 6.10a)***
C5	M	28.00	CI	Sudden	Chronic Moderate to focally marked Multifocal Mature fibrous and fibrofatty tissue	Mild Perilesional	None	Multifocal Random	Mild Lesion associated Mixed	None	Moderate to marked Multifocal

C7	M	21.87	CI	Sudden	Chronic-active Moderate to focally severe Multifocal Random Mature fibrous, fibrofatty and granulation tissue	Mild Perilesional	Mild Perilesional	None	Moderate Lesion associated Mixed	Acute Multifocal Random	Moderate to focally marked Diffuse
C8	M	39.42	EX	External	Chronic Moderate to marked Multifocal Random Mature fibrous and fibrofatty tissue	Mild Perilesional	None	None	Lesion associated, mild, mixed Patchy/scattered minimal to mild, perivascular and disseminated, mixed (mixed, mainly lymphocytic)	Acute Multifocal	Moderate diffuse to focally marked
C9	F	32.02	EX	External	Chronic-active Mild to focally moderate Multifocal Random Mature fibrous, fibrofatty and granulation tissue	None	Minimal to mild Perilesional	None	Minimal to mild Lesion associated Mixed Focal mild lymphocytic, epicardial	Acute Multifocal	Mild Multifocal
C15	F	46.32	IN	Euthanasia	Chronic Mild to moderate Multifocal Random Mature fibrous tissue	Mild Perilesional	Mild Perilesional	None	Minimal to mild Lesion associated Mixed	None	Mild Diffuse
C16	M	52.37	UK	Euthanasia	Chronic Mild Multifocal Mature fibrous and fibrofatty tissue	None	None	None	Minimal Lesion associated Mixed	Minimal to mi	None
C21	F	58.76	MI	Euthanasia	Chronic-active Mild to moderate Multifocal	Mild Perilesional	Minimal to mild Perilesional	Focal Perilesional	Minimal to mild Lesion associated Mixed	Acute Multifocal	Mild to moderate Diffuse

					Mature fibrous tissue, fibrofatty and granulation tissue				Patchy/scattered, mild, mixed, perivascular & disseminated		
C22	M	21.63	DI	Euthanasia	Chronic Mild to moderate Multifocal Random Mature fibrous and granulation tissue	Mild Perilesional	Minimal to mild Perilesional	None	Minimal to mild Lesion associated Mixed	Acute Multifocal	Mild to focally moderate Multifocal

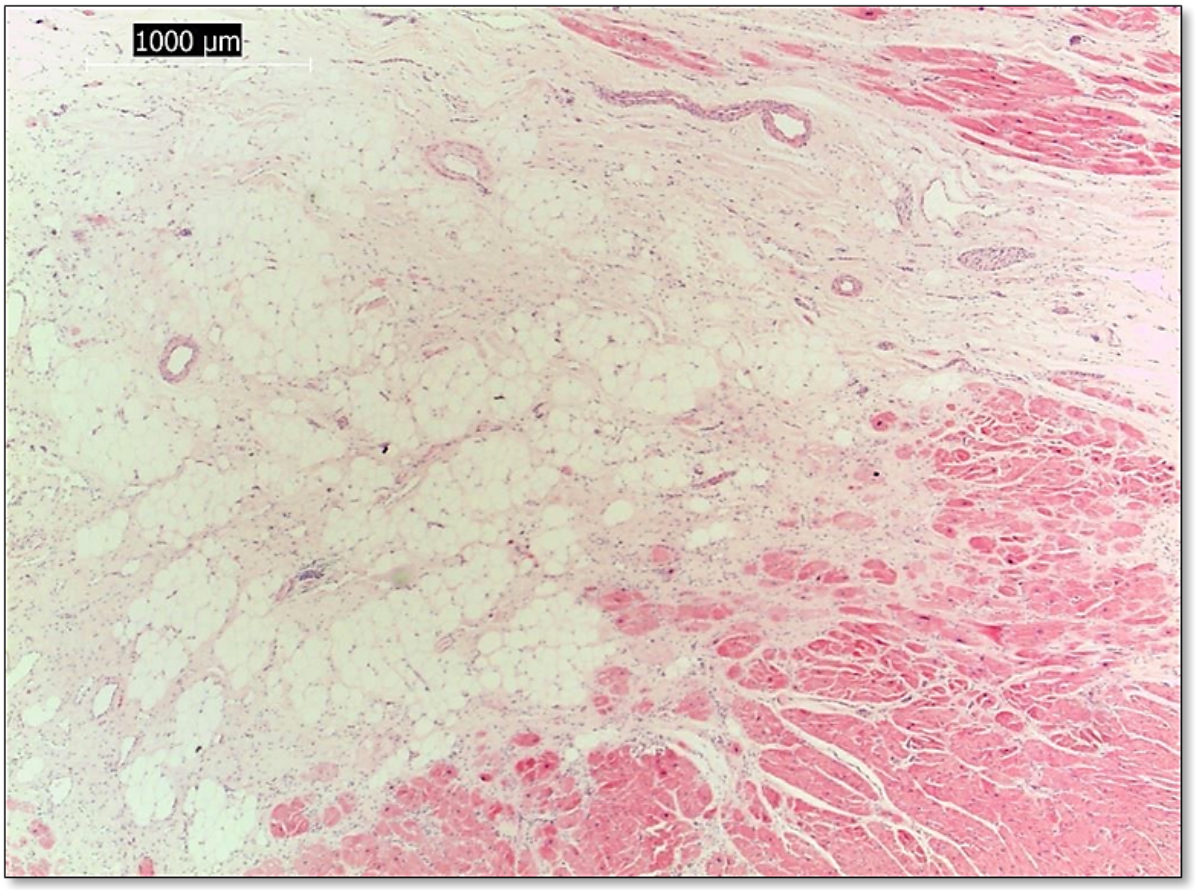


Figure 6.10a: Image showing low magnification H&E stained left ventricular myocardial section of chimpanzee heart affected by idiopathic replacement myocardial fibrosis.

This image demonstrates the extent of the myocardium that has been replaced by fibrofatty tissue. These areas can be further appreciated following special staining (see Figure 6.10b)

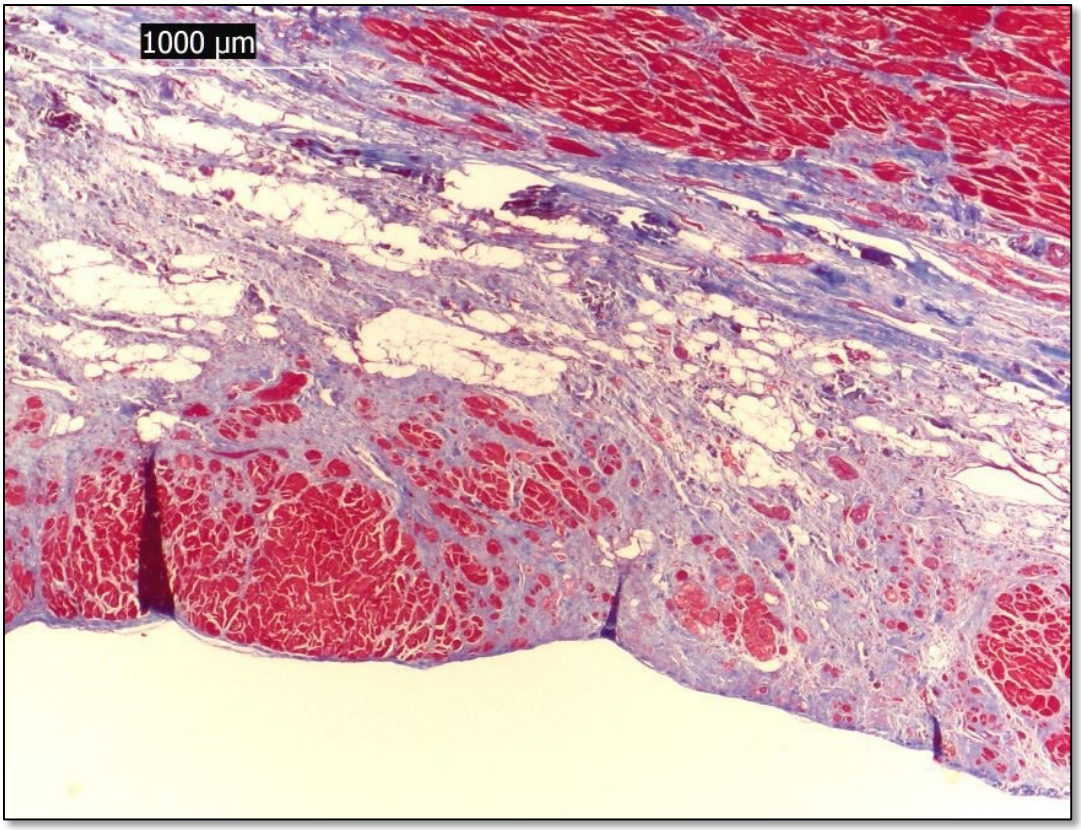


Figure 6.10b Image showing Masson's Trichrome stained left ventricular myocardial section of chimpanzee heart affected by idiopathic myocardial fibrosis.

The normal myocardium appears red whereas the collagen content of the scar tissue is stained blue. The white areas contained within these blue areas are accumulations of adipose tissue.

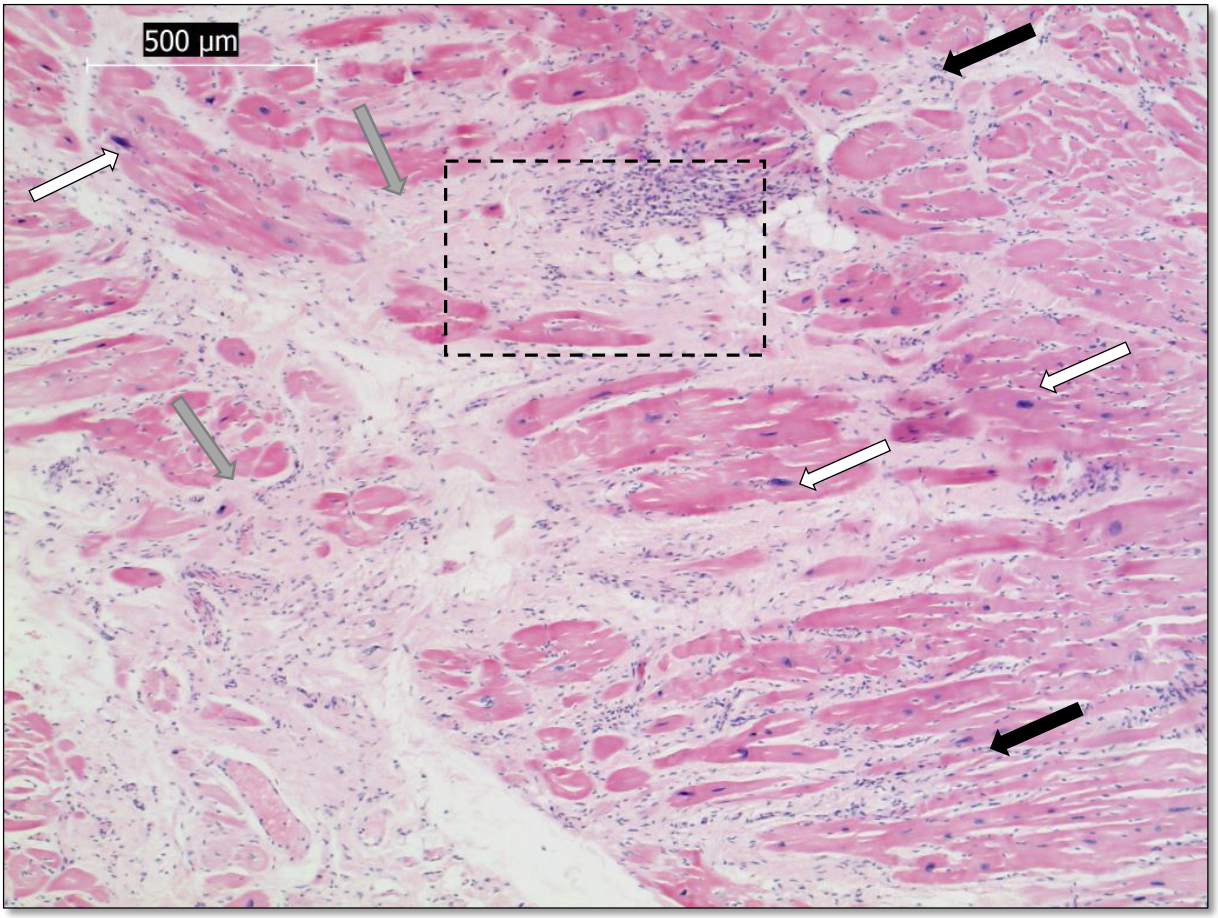


Figure 6.11a: Image showing H&E stained left ventricular myocardial section of chimpanzee heart affected by chronic-active idiopathic (interstitial and replacement) myocardial fibrosis.

Note the presence of both interstitial (black arrows) which often merges with replacement (grey arrows) fibrosis. Surrounding or entrapped myocytes show anisokaryosis and anisocytosis (white arrows), suggestive of reactive hypertrophy. The area highlighted in the dashed box is magnified and shown in Figure 6.11b.

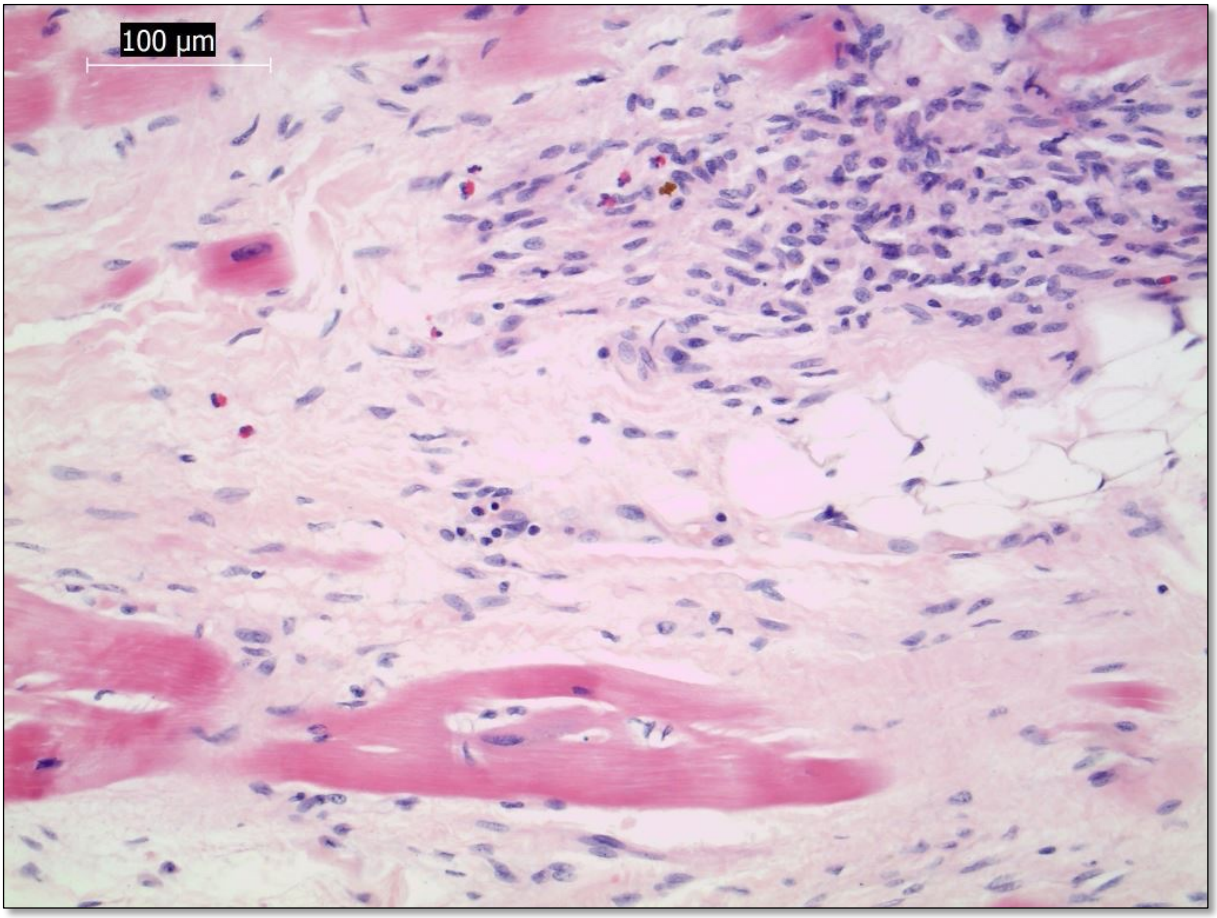


Figure 6.11b: Magnified image showing H&E stained left ventricular myocardial section of chimpanzee heart affected by chronic idiopathic myocardial fibrosis and the involvement of a leukocytic infiltrate

Note the presence of fibrous and fibrofatty tissue, as well as infiltration of lymphocytes, histiocytes and eosinophils

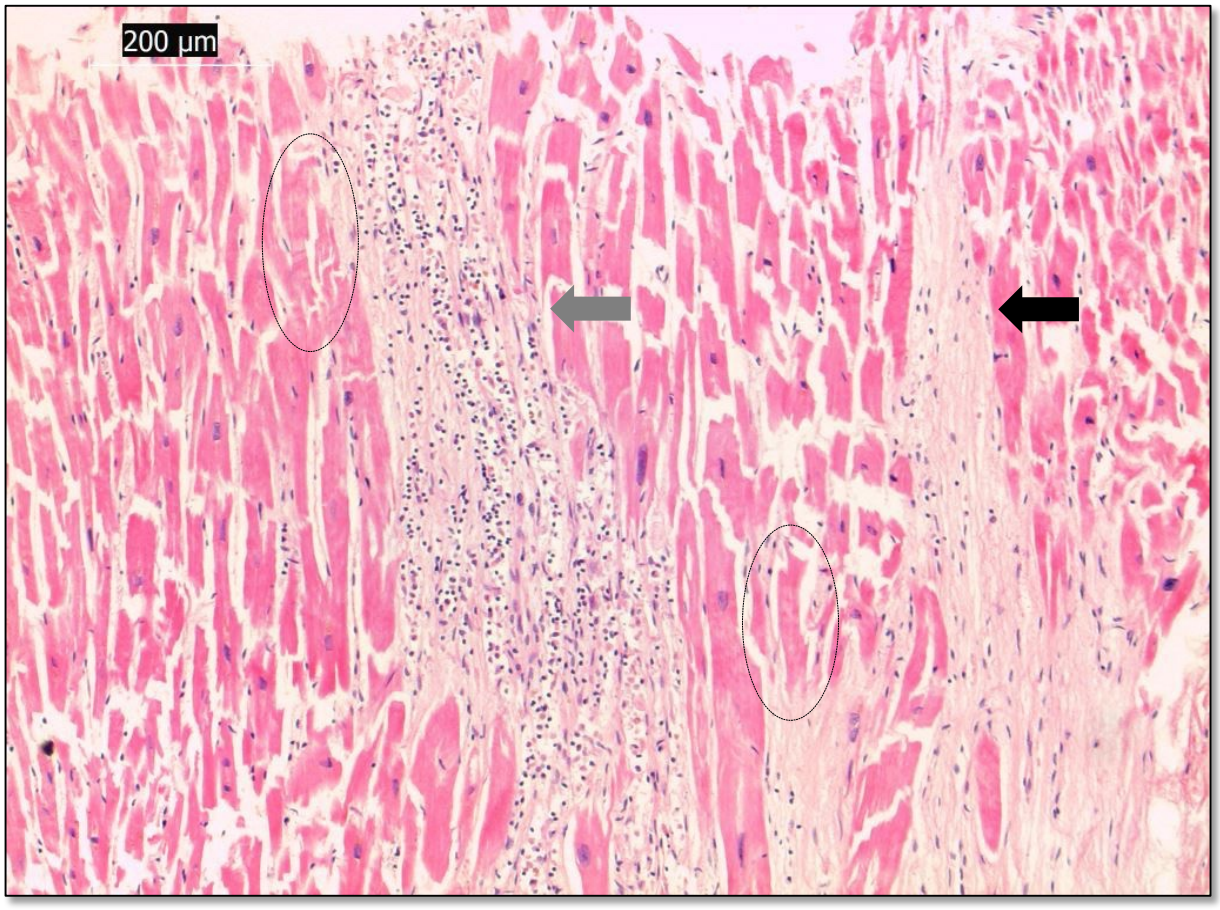


Figure 6.12 Image showing H&E stained left ventricular myocardial section of chimpanzee heart affected by chronic-active idiopathic myocardial fibrosis and acute degeneration

Note the area of mature replacement fibrosis (right side of the picture, black arrow); the subacute area of granulation tissue formation with a prominent leukocytic infiltrate (mid to left side of the picture, grey arrow) and; the presence of acute myocardial contraction bands (acute degeneration) adjacent to these two areas (circled)

6.3.5 Macroscopic examination: quantitative data analysis

Only quantitative data collected from fully grown (sub-adult, adult and aged) chimpanzees were included in statistical analysis. Data from juvenile chimpanzees (n=1) and all bonobos (n=5), gorillas (n=5) and orangutans (n=2) were excluded due to small sample size.

6.3.5.1 Chimpanzee vs human

Average heart weights for the chimpanzees studied were higher than those expected in humans, even when corrected for bodyweight (combined and male vs female) (Table 6.4). Left ventricular wall thickness was also found to be higher among chimpanzees than in humans (right ventricular wall thickness was similar). Mitral, aortic and pulmonic valve circumferences were similar, whereas tricuspid valve circumference was slightly smaller in chimpanzees than in humans.

6.3.5.2 Male vs female

The mean bodyweight of males included in this study were significantly higher than the females (p=0.0002). Mean heart weights (fresh and fixed), circumference and length were also higher among males, whereas heart weight to body weight ratios were lower (these differences were not statistically significant). Absolute chamber wall thicknesses were similar between the sexes, however, the ratio between left and right ventricular wall thickness was higher among females (5.4:1) than males (4.26:1) (p=0.0383) Male sex was also associated with an increase in mitral and tricuspid valve circumference although this difference was not found to be statistically significant.

6.3.5.3 Cause of death: cardiovascular vs other

The average bodyweight of those animals dying due to cardiovascular disease was significantly higher (p=0.0319) than that of those dying due to other causes (likely confounded by the difference in male to female bodyweights). Heart weight (fresh, p=0.0089; fixed p=0.0241) were also significantly higher in those animals which died due to cardiovascular disease as opposed to another cause. Heart weight to body weight (HW:BW) ratios were also higher among these animals, although this difference was not found to be statistically significant. Death due to cardiovascular disease was associated with an increase in circumference (p=0.0071) and length (p=0.028). It was also associated with an increase in right ventricular (p=0.005), left ventricular (p=0.6785) and interventricular septal wall (p=0.7688) wall thickness, and decrease in left to right ventricular wall thickness ratio (p=0.0023). Mean valve circumferences were higher among the group that died due to cardiovascular disease, however, this difference was not statistically significant.

6.3.5.4 Diagnosis: idiopathic myocardial fibrosis vs other

The presence of idiopathic myocardial fibrosis (as opposed to no, or any other cardiovascular condition) was associated with increased: heart weight (fresh and fixed), circumference, length, right ventricular wall thickness and all valve circumferences. The mean body weight of those affected was also higher than those which were unaffected. However, none of these differences were found to be statistically significant.

Table 6.4 Macroscopic quantitative data relating to sub-adult, adult and aged chimpanzee hearts which underwent post-mortem examination as part of this study
Data are combined and divided by sex, cause of death and according to whether idiopathic myocardial fibrosis was diagnosed following histopathology examination

		ALL COMBINED	BY SEX			IDIOPATHIC MYOCARDIAL REPLACEMENT FIBROSIS			CAUSE OF DEATH		
			Male	*Female*	*P value*[1]	*Diagnosed*	*Not diagnosed*	*P value*[1]	*Circulatory*	*Other*	*P value*[1]
Number of animals *(n)*		21	15	6	-	11	10	-	9	12	-
Bodyweight *(kg)*		55.17 ± 9.68	58.74 ± 9.01	46.25 ± 3.68	0.0002***	57.89 ± 10.41	52.18 ± 8.29	0.1789	60.29 ± 8.58	49.0 (40.3; 69.5)	0.0319*
Heart weight *(g)*	***Fresh***	390.3 ± 97.11	413.2 ± 99.26	349 ± 87.37	0.2401	397.4 ± 115.5	380.8 ± 75.18	0.7517	469.4 ± 58.64	346.3 ± 86.73	0.0089**
	Fixed	415.9 ± 164.7	435.3 ± 181.8	373.2 ± 125.5	0.4452	425.2 ± 156.1	403.9 ± 187.1	0.8120	545.5 ± 160.8	338.1 ± 113.6	0.0241*
HW:BW *ratio (%)*	***Fresh***	7.03 ± 1.80 (0.71%)	6.66 ± 1.33 (0.70%)	7.70 ± 2.46 (0.75%)	0.4184	6.71 ± 1.56 (0.69%)	7.47 ± 2.15 (0.73%)	0.4822	7.55 ± 0.67 (0.78%)	6.75 ± 2.19 (0.71%)	0.3348
	Fixed	6.29 (3.7; 12.93) (0.75%)	6.98 ± 2.70 (0.74%)	8.32 ± 2.97 (0.81%)	0.4165	7.29 ± 2.54 (0.73%)	7.54 ± 3.23 (0.77%)	0.8637	8.52 ± 2.73	5.74 (3.7; 11.67)	0.0727
Circumference *(cm)*		27.01 ± 3.52	27.42 ± 3.52	26.12 ± 3.76	0.5335	27.80 ± 3.97	26.0 ± 2.81	0.3067	29.82 ± 1.74	25.74 ± 3.42	0.0071**
Length (*cm)*		8.43 ± 1.47	8.66 ± 1.57	7.83 ± 1.08	0.2812	8.53 ± 1.66	8.33 ± 1.35	0.8041	9.53 ± 1.48	7.7 ± 0.95	0.028*
LV wall thickness *(cm)*		1.55 ± 0.28	1.55 ± 0.32	1.55 ± 0.21	0.9753	1.52 ± 0.33	1.59 ± 0.23	0.6023	1.59 ± 0.34	1.53 ± 0.24	0.6785
IVS wall thickness *(cm)*		1.64 ± 0.29	1.65 ± 0.33	1.62 ± 0.17	0.8027	1.61 ± 0.19	1.67 ± 0.38	0.6928	1.66 ± 0.37	1.62 ± 0.23	0.7688
RV wall thickness *(cm)*		0.3 (0.2; 0.5)	0.3 (0.2; 0.5)	0.3 ± 0.09	0.1813	0.38 ± 0.10	0.3 (0.2; 0.5)	0.1921	0.45 (0.3; 0.5)	0.3 (0.2; 0.4)	0.005**
LV:RV wall thickness ratio		4.62 ± 1.12	4.26 ± 1.02	5.40 ± 0.96	0.0383*	4.20 ± 1.18	5.09 ± 0.88	0.0792	3.80 ± 0.77	5.22 ± 0.95	0.0023**
MV circumference *(cm)*		8.7 ± 0.98	9.1 (6.5; 10.2)	8.4 ± 1.04	0.2581	8.93 ± 0.93	8.41 ± 1.02	0.2858	9.1 ± 0.26	8.45 ± 1.19	0.1049
TV circumference *(cm)*		10.42 ±1.51	10.55 ± 1.39	9.4 (8.7; 13.4)	0.3547	10.98 ± 1.75	9.79 ± 0.90	0.0798	10.9 ± 0.99	9.4; (8.4; 13.4)	0.0533
AoV circumference *(cm)*		5.53 ±0.86	5.45 ± 0.90	5.76 ± 0.81	0.4966	5.63 ± 0.77	5.41 ± 1.01	0.6226	5.71 ± 0.77	5.42 ± 0.93	0.4764
PV circumference *(cm)*		6.24 ± 1.04	6.26 ± 1.14	6.2 ± 0.9	0.9129	6.41 ± 1.04	6.05 ± 1.09	0.4959	6.5 ± 1.08	6.1 ± 1.05	0.4774

[1] Unless denoted otherwise (with *), result is not statistically significant*; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001*

6.4 Discussion

This study utilised a standardised and consistent methodology to identify and describe various pathological lesions affecting the cardiovascular system of great apes. It also demonstrated the variety of conditions and/or lesions which can present histologically as myocardial fibrosis. By carrying out a very detailed macroscopic inspection and standardised histopathologic examination of a representative number of myocardial sections of consistent areas, aetiological explanations for the presence of the myocardial fibrosis in many of these cases could be identified or proposed (e.g. myocardial infarction, ARVC, left ventricular hypertrophy, sepsis). Without such detailed examinations being carried out, these might have been diagnosed with 'fibrosing cardiomyopathy' (FCM), the issues associated with which have already been outlined in Chapter 3 and in the introduction of this chapter.

For twelve chimpanzees, however, no such cause could be identified and these animals were therefore diagnosed with idiopathic myocardial fibrosis. Through more detailed analysis and correlation of histopathological and macroscopic qualitative and quantitative data gathered from this sub-group of animals, the key features of chimpanzee idiopathic myocardial fibrosis were further characterised and are hereby discussed.

6.4.1 Chimpanzee idiopathic myocardial fibrosis (IMF)

Macrosopically, the chimpanzee hearts affected by IMF were often found to exhibit pale pink to whitish epicardial and myocardial mottling. The multifocal to coalescing nature of this mild to moderate mottling suggests a different aetiopathogenesis from hypoxic myocardial infarctions, which are typically associated with more focally extensive or regional areas of intense pallor or redness, often emanating from a coronary artery or one of its major branches.

Nonetheless, the fact that such colour irregularities were also present in healthy hearts (fixation artefact) and those affected by other conditions or pathological changes, suggests that it is not a reliable specific indicator of myocardial fibrosis.

Despite some subjective reports of an impression of mild hypertrophy, rounding or dilation of the heart, these correlated poorly with objective measurements and histopathologic findings. In fact, IMF was not associated with any major structural or morphological changes. Many artefactual changes can give the impression of genuine pathological lesions: for example, myocardial rigor, which is particularly common among the elderly, is an agonal change associated with an increase in ventricular wall thickness and decrease in chamber diameter (Sheppard & Davies 1998). To the naked eye, this can easily be over-interpreted as hypertrophy, demonstrating the need to reinforce any such subjective impressions with objective measurements, such as heart weight. Whilst IMF was associated with a slight increase in heart weight (not statistically significant), this was not true of the HW:BW ratio. This demonstrates the importance of recording both the heart and body weights and of interpreting them in relation to one another, when assessing heart mass. It should also be borne in mind, that some structural changes might in fact be physiological rather than pathological. A good example of this is physiological cardiac hypertrophy associated with exercise in athletes (McMullen & Jennings 2007). The gold standard for identifying true pathological lesions and distinguishing them from physiological or artefactual change, therefore, is histopathology.

Histologically, multiple randomly distributed areas of myocardium were replaced by myocardial fibrosis (replacement fibrosis). There was also a mild to marked, diffuse increase in connective tissue within the inter-myofibre space (interstitial fibrosis) in all of the hearts examined. Previous reports of myocardial fibrosis published within the literature refer only/mostly to interstitial fibrosis (Baldessari et al. 2013; Lammey, Baskin, et al. 2008; Varki

et al. 2009). Distinguishing between these two distinct patterns of fibrosis is very important, as the pathogenesis behind their development differ greatly (as reviewed by Mewton et al. 2011). However, given than interstitial fibrosis can ultimately lead to replacement fibrosis in the later stages of disease, it is possible that both could be part of the same disease process in great apes (as reviewed by Mewton et al. 2011). The notion that interstitial fibrosis might in fact come first, is further supported by it being only minimal to mild and associated with only mild replacement fibrosis in the youngest animal examined (C1; 9.98 years) and yet moderate to severe, and associated with more marked and extensive replacement fibrosis in many of the older animals.

The presence of myocardial fibrosis and associated changes (e.g. cellular infiltration, peri-lesional reactive myofibre hypertrophy) and absence of the hallmark features of any other common cardiovascular conditions (e.g. valve degeneration or stenosis, coronary artery disease, gross hypertrophy or dilation) suggests that IMF in chimpanzees is most comparable to a condition with the same name in humans.

6.4.2 Idiopathic myocardial fibrosis in people

(Human) idiopathic myocardial fibrosis has been cited as the cause of between 1-3% of sudden cardiac death cases (Bowker et al. 2003; Davies 1999; John et al. 2004). It has been defined, as the macroscopic or microscopic scarring in subjects who do not have cardiac failure but who present with either ventricular tachycardia and/or sudden death (John et al. 2004; Sheppard & Davies 1998). The lesions themselves are very similar in appearance to that seen in arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D). ARVC is characterised by progressive myocyte loss, fibrofatty replacement and the presence of chronic inflammatory infiltrates (Basso et al. 1996; Corrado et al. 1997). As the name suggests, ARVC

predominantly affects the right ventricle, whereas IMF in humans reportedly affects the left ventricular myocardium (John et al. 2004). Given that left dominant as well as biventricular forms of ARVC have more recently been described, it has been suggested that all three of these pathological presentations are in fact part of the same disease spectrum and, as such, the broader term 'arrhythmogenic cardiomyopathy' is now being used (Pinamonti et al. 2014; Sen-Chowdhry et al. 2007; Sen-Chowdhry et al. 2008). Like IMF in chimpanzees, human patients with all three (left dominant arrhythmogenic cardiomyopathy (LDAC), IMF, ARVC) conditions are at risk of life threatening arrhythmias and sudden death.

6.4.3 Chimpanzee idiopathic myocardial fibrosis: searching for a cause

Given the similarities described, it must be considered, that the IMF described in this study and by others to affect great apes is in fact part of the same or a similar disease spectrum as IMF, LDAC and maybe ARVC in people. Each of these have been associated with mutations in the genes encoding desmosomal proteins and follow a strong familial pattern (Delmar & McKenna 2010; Sen-Chowdhry et al. 2008;). Combining studbook and mortality data to assess for patterns of inheritance as well as screening of affected animals for such genetic mutations is therefore an important next step for great ape cardiovascular disease aetiopathogenesis investigation. For a primary/inherited cardiomyopathy, however, the prevalence is very high. For example, IMF was diagnosed in 55% (n=12/22) and was the cause of death in 42% (n=5/12) unrelated chimpanzees. By comparison, ARVC/D is reported to affect 1:1000 to 1:1250 of the general population (Peters 2006). It must also, therefore, be considered that the fibrosis observed at post-mortem examination is merely the end-product of a reparative process following cardiac damage by another insult. All stages of the IMF process (myocyte degeneration, granulation tissue formation, chronic fibrosis) were observed. The chronic-active

nature of the lesions therefore suggests that the cardiac damage involved is either continual or at very least, repeated or periodic. The source of this cardiac insult, however, currently remains unclear.

The pattern, distribution and type of myocardial fibrosis might be able to offer clues as to the aetiopathogenesis (as reviewed by Mewton et al. 2011). For example, interstitial fibrosis can be infiltrative, as in amyloidosis and Anderson Fabry's disease, but can also be reactive and stimulated by another pathological process (e.g. in hypertension, diabetes mellitus, idiopathic DCM, HCM, pressure/volume overloading, chronic kidney disease) (Asbun & Villarreal 2006; Heymans et al. 2015; López et al. 2008; Mewton et al. 2011). Interstitial fibrosis can also occur as a normal feature in myocardial ageing (see later). Before the cardiac interstitial and replacement fibrosis reported here in chimpanzees can be defined as being truly idiopathic, therefore, an association with systemic co-morbidities should be ruled out. Unlike interstitial fibrosis, replacement fibrosis occurs when myocytes are damaged or destroyed. In people, the most common cardiovascular condition and cause of myocyte damage or death, is ischemic heart disease. Fibrosis resulting from ischemic (hypoxic) myocardial damage typically preferentially affects the sub-endocardial myocardium (Geer et al. 1980). However, the IMF lesions identified in this study were present throughout the myocardium, showing no particular predilection for the sub-endocardial, sub-epicardial or mid-myocardial regions. This further supports the conclusions presented in Chapter 3 of this thesis, and by others (Varki et al. 2009), that ischemic heart disease is not a significant issue among great apes nor the cause of the fibrosis being described. Although interesting, this is perhaps of little surprise, given that these animals are not exposed to the risk factors typically associated with coronary artery disease in people (e.g. tobacco smoke, alcohol, high fat diets). Other, non-ischemic, causes of myocardial fibrosis must therefore be considered.

The consistency with which cellular infiltration was identified, lends itself to a possible inflammatory theory behind the aetiopathogenesis of IMF in chimpanzees. The cardiac inflammation might simply be reactive and occurring in response to myocyte death. Indeed, intermittent waves of myocyte apoptosis and reactive myocarditis have been reported to form part of the natural disease course in ARVC (Thiene et al. 2000; Yamaji et al. 2005; Sen-Chowdhry et al. 2008). It should also be considered, however, that the cardiac inflammation is occurring in response to inflammation elsewhere in the body. However, if this were the case, the cellular infiltration would be expected show a more generalised or perhaps perivascular distribution and not to persist once chronic scarring (fibrosis) had occurred.

In human medicine, a wide array of infectious (viral, bacterial, fungal, rickettsial, helminthic and protozoal) causes of acute myocarditis have been identified (Dennert et al. 2008). Acute myocarditis typically presents clinically as non-specific or even flu-like illness (for example: fever, malaise), before more cardiac specific symptoms such as chest pain, dyspnoea, palpitations and cardiac failure develop (Andréoletti et al. 2009; Dec et al. 1985; Kearney et al. 2001; Mahrholdt et al. 2006; Sheppard & Davies 1998). Most cases of acute myocarditis resolve without clinically significant consequence. However, some patients (10-20%) go on to develop more chronic cardiac inflammation, which is typified by mononuclear cell infiltrate, myocardial necrosis and fibrosis and is associated with left ventricular systolic dysfunction. The chronic persistence of viral DNA/RNA even beyond this has been cited as a cause of dilated (DCM) and arrhythmogenic right ventricular (ARVC) cardiomyopathy (Andréoletti et al. 2009; Kawai 1999; Kearney et al. 2001). Given that, in captivity, the contact between great apes and their human carers is regular and close, cross exposure to infectious pathogens is highly likely. The potential that viral infection +/- persistence is involved in the pathogenesis of great ape cardiovascular disease and specifically myocardial fibrosis, is therefore an important area for investigation in the future.

Not all causes of myocarditis, however, are infectious (Feldman & McNamara 2000; Sheppard & Davies 1998). The role that other (toxic, immune-mediated, environmental, nutritional) factors might play in causing or predisposing zoo-housed great apes to cardiac inflammation must also therefore be considered. For example, receiving significant attention over recent years for its immunomodulatory benefits is vitamin D (Guillot et al. 2010), deficiencies in which have been linked with an increased risk of cardiovascular, and specifically myocardial, disease (Anderson et al. 2010; Holick 2004; Pilz et al. 2010). Being endemic to central Africa and South East Asia, wild great apes are exposed to high levels of sunlight and therefore also vitamin D. The amount of sunlight to which zoo-housed great apes exposed will inevitably be much lower, especially throughout periods of winter housing, the health effects of which warrant further investigation. Wild great apes also ingest a diet which is rich in fruit, plants, nuts and seeds. Amongst other things, these foods are known to be a good source of salicylic acid (Hare et al. 2003). As a derivative of aspirin, the health benefits and specifically the anti-inflammatory effects of dietary salicylic acid have long been studied (Amann & Peskar 2002; Higgs et al. 1987; Rinelli et al. 2012). Given that great apes in the wild would ingest large volumes of plant material that cannot be replicated in the zoo environment, it might be hypothesised that a deficiency in salicylic acid might also therefore be involved in the development of cardiac inflammation.

One final consideration, is whether the changes observed are in fact a normal feature of myocardial ageing. This notion might be supported by the fact that such changes were identified in all chimpanzees >20 years old examined as part of this study. Ageing is associated with an increase in connective tissue deposition (reactive fibrosis) in the interstitial and perivascular spaces (Biernacka & Frangogiannis 2011; Heymans et al. 2015; Jellis et al. 2010; Kajstura et al. 1996; Macri et al. 2012; Olivetti et al. 1991; Sangaralingham et al. 2011). Whilst ageing is also associated with apoptosis and necrosis (Kajstura et al. 1996; Olivetti et al. 1991),

and an overall reduction in cardiomyocyte number, this will likely occur on a sporadic, single cell basis. The multifocal, sometimes focally extensive areas of reparative/replacement fibrosis described in this chapter, therefore, are very unlikely to be a normal and inevitable effect of myocardial ageing. This is also validated by the fact that similar changes were also seen in animals as young as 10 years of age and yet were not present feature, for example in the elderly female gorilla hearts, nor the 37-year-old male bonobo heart examined.

6.4.4 Quantitative data analysis results

From the data collected in this study, it is not possible to state whether the observed correlation between increased heart size (weight, circumference and length) and risk of death due to cardiovascular disease was cause or effect. The possibility that increased heart size, and indeed also bodyweight, are risk factors for cardiovascular disease related death in chimpanzees warrants further investigation. It is possible however, that some, if not all of these differences can in fact be accounted for by differences in heart size and bodyweight between male and female chimpanzees. The increased thickness of the right ventricular wall identified in those animals dying due to cardiovascular disease, is also of interest. Unlike overall heart size, this figure is unlikely to be confounded by sex variation, as the left to right ventricular wall thickness was in fact higher among females than males. Although not found to be statistically significant, the right ventricular wall thickness of IMF affected hearts was found to be higher, suggesting therefore that any such differences in wall thickness are associated with infiltrative/hypertrophic as opposed to dilative changes. Analysis of a larger dataset is, however, required to substantiate these findings and improve their statistical significance.

The observation that heart weight and left ventricular wall thickness were both higher among the chimpanzees included in this study than would be expected in humans of similar body weight was also very interesting. It may of course simply be the case that this variation is to be

expected between the species. However, the possibility that the chimpanzee heart is perhaps comparable to that of a human athlete must be considered. Given the propensity for cardiac arrhythmias and sudden cardiac death both among both athletes (Maron & Pelliccia 2006) and chimpanzees (Doane et al. 2006; Lammey, Lee, et al. 2008; Chapter 3), any such similarities would be of particular relevance and interest.

6.4.5 Limitations of the study

Whilst the largest dataset of its type to date, this study was still limited by the number of animals included. This is especially true of bonobos, gorillas and orangutans for which the number of heart samples collected were comparatively much smaller than for chimpanzees. The findings could therefore be validated and conclusions strengthened by the future examination of additional samples, especially from these taxa.

In some cases, the findings were also limited by the quality and completeness of the sample received. Many had undergone prior sectioning by the zoological collection of origin for their pathologist. However, the proportion of excellent quality samples available is likely to increase over time as awareness and use of the basic examination protocol developed in Chapter 4 (Appendix 10) is further enhanced. Nonetheless, some zoological collections will desire that the hearts are examined in-house or by their pathologists prior to being submitted to this research project for further sectioning and examination. In these instances, it is hoped that referring them to the detailed protocol (Appendix 11) will not only help to improve not only the quality of the individual diagnosis but also still permit comparison between the information and data collected as part of this study.

THESIS CONCLUSIONS

I Zoo-housed great ape health, disease and mortality

The health of zoo-housed great apes is important from not only the perspective of each individual animal's welfare but also the wider conservation of the species. In the first ever systematic review of the great ape literature (Chapter 1), 189 published articles relating to great ape morbidity and mortality were reviewed. From this, it was concluded that there was a critical need for a robust, widespread and up-to-date review of zoo-housed great ape mortality especially among the European population. Such a review of data relating to 681 great ape deaths was carried out and is outlined in Chapter 2 of this thesis. The post-mortem data collected and analysed as part of this mortality study, has since been collated into a centralised electronic database, to which access is controlled through the EAZA great ape TAG and its veterinary advisors. The availability of such a rich dataset has the potential to be of great value to researchers wishing to study any aspect of zoo-housed great ape disease and mortality in greater detail.

Great differences and inconsistencies in the approach to post-mortem examination and investigation between zoological collections were found to exist. A series of recommendations about how such examinations, and the information collected from them could be improved and better utilised were therefore made. The planned adoption of these recommendations by the EAZA Great Ape TAG will facilitate and greatly enhance future reviews of this type, and enable consistent monitoring of patterns of mortality within these populations in the future.

The conclusions drawn from the mortality study also have the potential to inform and influence the future day-to-day management and veterinary care of zoo-housed great apes. As well as identifying particular diseases of interest or concern, they highlight a need for the provision of geriatric healthcare, and a greater understanding about chronic and age-related diseases. Despite the existence of an ageing zoo-housed population of great apes, perinatal and infant

mortality was still found to be a frequent occurrence. Simply studying the total number of such deaths that occur during a given time period, however, can be misleading. Perinatal and infant mortality rates should be interpreted alongside birth +/- pregnancy rates as well as whole population numbers, to provide better perspective on their potential impact on the overall growth and sustainability of the population. Such analyses and population forecasting are carried out by the individual species coordinators and studbook holders on a regular basis. There is the potential, therefore, for studbook and mortality data to be combined to predict the potential impact of any given cause of mortality on long-term population sustainability.

Specific disease groups were identified by this review as requiring further study into their aetiopathogenesis, due to the frequency with which they were associated with mortality. These included: respiratory disease, especially among bonobos and orangutans, and disorders of the digestive system in gorillas. Diseases of the cardiovascular system were also identified as a significant cause of death especially among adult zoo-housed gorillas, bonobos and chimpanzees. Despite this, and the large number of articles on this topic which were found to exist in the current literature (Chapter 1), understanding about the epidemiology, pathogenesis and indeed also diagnosis of cardiovascular disorders in great apes was found to be poor. These three topics were therefore the focus of the rest of this thesis and the main findings discussed in greater detail below.

II Great ape cardiovascular disease

II.I Great ape cardiovascular disease: epidemiology

Studying the distribution and patterns of cardiovascular disease within these populations provided insight into risk factors that might be associated with its pathogenesis (Chapter 3). For example, male sex was identified as a risk factor for death due to cardiovascular disease. The mechanisms involved in this male predisposition to cardiac disease would be a fascinating topic for further investigation. An interesting study might, for example, involve comparing the prevalence of cardiovascular disease, or frequency with which it is associated with mortality, between entire versus castrated male animals. Should differences be identified, it would be of benefit to also try to study the role that testosterone levels play in the pathogenesis of great ape heart disease. It might be, for example, that the increase in sex hormone production (e.g. at sexual maturity) or decrease (as occurs with age) coincides with the onset of cardiovascular disease. In addition, the role of testosterone is likely, not only to differ with the animal's contraceptive status but also its social position. Given that social status and stress are also closely related (Sapolsky 2005), the interplay between all of these factors and cardiovascular disease risk would also be an interesting topic for ongoing longitudinal study.

Increasing age was also identified as a risk factor for cardiovascular disease among great apes. In people, age is associated with an accumulation of cardiovascular disease associated risk factors (the so-called life course perspective of chronic disease) and therefore an increased rate of mortality (Aboderin et al. 2002; Hobel & Arora 2010; Kuh & Shlomo 2004). It is likely, therefore, that a similar situation occurs in ageing great apes. Nonetheless, chronic cardiovascular lesions were identified in animals as young as 10 years of age (Chapters 2 & 6). It was also found to be absent in the hearts of other adult and even elderly great apes (Chapter 6), suggesting that the development of cardiovascular disease is not an inevitability nor is it

fully explained by ageing alone. The notion that cardiovascular disease is merely an inevitable feature of ageing was also contradicted by the finding that it occurred far less frequently in European zoo-housed orangutans than the other great ape taxa, despite their slightly longer life expectancy. This suggests that cardiovascular disease is in fact multifactorial, being caused by several factors that, together, are associated with disease development and ultimately death.

II.II Great ape cardiovascular disease: pathogenesis

The development of a protocol for the consistent and standardised post-mortem examination of the great ape heart (Chapter 4) has improved the quality of pathological description and diagnosis of cardiovascular pathological lesions. All zoos submitting samples for detailed examination using this protocol have received a full written report detailing the macroscopic and histopathologic findings. The report provides far greater detail than those typically provided by most veterinary pathologists. They also contain a final comment in which the findings are interpreted; opinions relating to the significance and possible aetiologies of the pathological changes are given. From a research standpoint, the use of this protocol has resulted in the collection of comparable and good quality biological samples for future research. It also enabled the collection and subsequent analysis of a large amount of data as part of a study investigating great ape cardiovascular pathology in greater detail (Chapter 6). This study, which involved the detailed macroscopic and histopathologic examination of 34 great ape hearts, has vastly improved perspective about normal versus abnormal findings encountered in post-mortem examination of the great ape heart. It has allowed, for the first time, the meaningful comparison between the pathological findings in hearts collected by various individuals from numerous institutions across Europe and Africa. The findings of the study highlight the importance of carrying out a very detailed examination, which should include

histopathology even in the absence of macroscopic changes. They also demonstrate the value in taking numerous sections for histopathology, as this allows a true appreciation of the extent and distribution of the pathological lesions to be gleaned. It is hoped that, through publication of the post-mortem protocol itself as well as the research study findings presented in Chapter 6, this work will help to improve the approach to the post-mortem investigation and diagnosis of great ape cardiovascular disease worldwide.

Fibrosis is a common finding in post-mortem examination of the great ape heart. Its possible causes, however, are many and its presence is merely a non-specific indicator of cardiac insult or injury. The in-depth examination of myocardial sections from numerous pre-determined locations in the pathology study (Chapter 6) was shown to prevent over-interpretation of the presence of myocardial fibrosis by allowing for cases of localised damage (caused for example by an ischemic insult) to be distinguished from more widespread degenerative disease. Detailed description and characterisation of the pattern and distribution of the lesions also allowed for certain possible aetiologies to be identified or discounted. Those animals for which no apparent cause for the myocardial fibrosis could be identified, however, were diagnosed with idiopathic myocardial fibrosis (IMF) and their histological features reviewed in greater detail. The use of a standardised approach to their examination and consistent terminology in their description, allowed for meaningful comparison between the IMF affecting great ape hearts with similar cardiovascular conditions reported and diagnosed in humans. This, in turn, has also allowed for evidence based speculation about the possible aetiopathogenesis of IMF, specifically in chimpanzees. For example, it has highlighted the similarities that exist between chimpanzee IMF and 'human' arrhythmogenic cardiomyopathies such as ARVC, LDAC and IMF. Given the strongly familial basis of these cardiomyopathies in humans, investigation into whether IMF is in fact a primary cardiomyopathy with a genetic basis in chimpanzees would be a fascinating next step for any research in this field. Suggested studies, therefore, might

include for example: combining studbook and mortality data to assess for patterns of inheritance, or screening affected animals for known genetic mutations. Such studies are now possible, thanks to the existence of a rich database of mortality information, created as part of the work outlined in this thesis.

It is possible, however, that myocardial fibrosis and the cardiomyopathy with which it is associated, is not caused by inherited genetic mutations. It might, in fact, be the end-stage result of another pathological process. The consistency with which cellular infiltration was identified, lends itself to a possible inflammatory theory behind the aetiopathogenesis of IMF in chimpanzees. Whether the inflammation is merely a reaction to myocyte death or is, itself, part of the aetiopathogenesis, is not yet clear. Although the results are not presented in this thesis, a pilot study designed to investigate the possibility that the presence or persistence of an infectious (likely viral) pathogen is responsible for the observed inflammation and scarring has been initiated. The role that other environmental and dietary factors (e.g. vitamin D, salicylic acid content), might also play in predisposing animals to, or perpetuating, cardiac inflammation should also be examined. Further information about the involvement of inflammation in the pathogenesis of chimpanzee IMF might also be gleaned for example by: characterising the immune response (performing immunohistochemistry to distinguish whether it is B- or T-cell mediated; identifying/quantifying the expression of certain inflammatory mediators such as tumour growth factor (TGF)-beta or; comparing the metabolomic and proteomic profile of tissues taken from affected and unaffected individuals. The latter, more complex, of these investigations has been made possible by the collection of fresh frozen as well as RNAlater preserved myocardial sections, due to the adoption of the protocol developed in Chapter 4 of this thesis. Furthermore, any metabolites or proteins identified as being involved in, or generated by, the pathological processes, also have the potential to serve as biomarkers of disease or even therapeutic targets in the future.

II.III Great ape cardiovascular disease: ante-mortem (clinical) diagnosis

Great apes affected by cardiovascular disease, and especially cardiomyopathies, typically do not show any signs of ill health, presenting only as cases of sudden or unexpected death (Chapter 3). This therefore suggests a need for pro-active screening to identify pre- or sub-clinically affected animals. Echocardiography is often considered the mainstay diagnostic modality in such screening but, even in familiar species, is prone to inter- and intra-observer variability (Pinedo et al. 2010; Thavendiranathan et al. 2013; Vignola et al. 1977). Its utilisation for the clinical assessment of great ape cardiac structure and function and especially research data collection has therefore been limited. The development of a protocol for the echocardiographic assessment of great ape cardiac structure and function (Chapter 4) has, however, improved and standardised the approach across European zoological collection.

The protocol is designed for use in animals which have been immobilised to allow safe handling and detailed examination. The accuracy and reliability of these diagnostic tests in assessing cardiac structure and function in anaesthetised animals under, however, is still under debate. The alpha-2-agonists in particular, are often said to prevent meaningful interpretation of echocardiographic findings and to predispose animals to peri-anaesthetic mortality (Brainard 2016; GAHP 2016a). The evidence for these claims, however, is limited. Data presented in Chapter 5 of this thesis in fact appears to suggest that, despite significant effects of haemodyamic parameters and anaesthetic quality, low-dose medetomidine for example, has minimal effect on echocardiographic measurements obtained from healthy chimpanzees. The conclusions drawn from this study suggest that the situation with regards the effects of alpha-2 agonists on the heart and their implications for cardiovascular disease screening, however, is not at all clear cut. Further studies investigating the effects of these and other anaesthetic agents

on the great ape cardiovascular system are therefore required. Such investigations, would be greatly enhanced by an ability to assess cardiac structure and function in an awake animal. Given the risks associated with anaesthesia of any patient with cardiovascular disease, the ability to diagnose or monitor heart disease without the need for anaesthesia, would also be of great clinical value. Several great apes housed in North American zoos have been trained for conscious echocardiographic and/or blood pressure assessment (GAHP 2016b). Methods for awake telemetry (ECG) assessment using purpose designed non-invasive probes have also been developed at Twycross Zoo as part of the work of the Ape Heart Project. Gathering further valuable information about the heart's electrical activity as well as continual monitoring of cardiac rhythm and heart rate variability, for example, has also been made possible by the placement of implantable loop recorders in a small number of chimpanzees and gorillas housed in zoos in both the USA and UK. Their more widespread use, however, is limited by their cost, the tendency for animals to interfere with and/or remove them, and ethical considerations relating to patient selection.

These issues, which limit our abilities to diagnose cardiovascular disease, are confounded by the fact that it is not yet clear, how many of these conditions manifest clinically. Data presented in Chapter 6 suggested that IMF, for example, is not associated with any significant changes in cardiac structure, meaning that the assessment for example of wall thickness on echocardiography is likely to be a poor indicator for the presence/absence of disease. It is possible, however, that measures of cardiac function (i.e. contractility, ejection fraction, tissue Doppler etc.) will be of greater use in identifying affected animals, although determining whether this is the case, requires further study. It might, in fact, be the case that IMF does not cause any detectable change in parameters measurable by echocardiography, therefore rendering it to be of little diagnostic use. If this is the case, attention must turn instead to identifying alternative diagnostic modalities which instead might be used in the identification

of affected animals. In human medicine, myocardial fibrosis can be detected and quantified using magnetic resonance imaging (MRI) (Mewton et al. 2011), however, the practical application of this in great ape medicine is limited. In humans, arrhythmogenic cardiomyopathies such as ARVC, IMF and LDAC have been associated with quite subtle depolarisation, repolarisation and conduction abnormalities as well as arrhythmias on ECG. These include: the presence of epsilon waves; localised prolonged of the QRS complex in the right precordial leads; inverted T waves in right precordial leads; bundle branch block ventricular tachycardia and; frequent ventricular extra systoles (Sen-Chowdhry et al. 2008; Mckenna et al. 1994). There are no published studies in which ECGs taken from zoo-housed great apes are screened for these such abnormalities. Although not included in this thesis, a standardised and consistent method for carrying out a 12 channel ECGs in great apes has been developed and is freely available on the Ape Heart Project website (Appendix 15). As for the echocardiography protocol discussed in Chapter 4, this guide was developed based upon recommendations for use in people. Its use has allowed for consistent and comparable, good quality, 12-channel ECG data to be collected as part of the routine health assessment of great apes at Twycross Zoo. This data can be used not only for the benefit of the individual undergoing screening, but also in research, again demonstrating the positive impact of the work carried out as part of this thesis.

Similarly, more than six years' data relating to the serum concentrations of the biomarkers brain natriuretic peptide (BNP) and cardiac troponin have also been routinely collected from great apes undergoing routine health assessment at Twycross Zoo. The degree to which the concentration of these biomarkers correlate with other ante-mortem as well as post-mortem findings, however, has not yet been studied; another potential study to be carried out in the future. Given that BNP and troponin are indicators specifically for increased wall stress/tension and myocyte injury respectively (see review by Archer 2003) it is possible that their levels may

not be affected for example in cases of IMF. More research might therefore instead be required to identify biomarkers specific to the fibrotic process (such as pro-collagen pro-peptides) occurring in these hearts (de Jong et al. 2011; López et al 2015). The main challenge that currently exists for all such studies, however, is our inability to confidently determine an animal's disease status. The only means by which affected and unaffected great apes can currently be distinguished from one another, is on histopathology, and therefore currently only at post-mortem. In the future, more invasive techniques such as endomyocardial biopsy which is used in human medicine, might also be used in great apes. Until then, a better appreciation for how IMF can be diagnosed in life is likely to come instead from studies correlating ante-mortem and post-mortem data collected from the same animals. The longitudinal monitoring of animals for this purpose has been made possible by the work outlined in this thesis: a wealth of good quality, comparable post-mortem data, clinical information and biological samples (heart tissue, blood, serum, urine) is now held at Twycross Zoo. As property of the Ape Heart Project, and therefore the EAZA Great Ape TAG, they are available for additional studies investigating the aetiology and pathophysiology of great ape cardiovascular disease in the future, for which they will be an invaluable resource.

II.IV Great ape cardiovascular disease: wild versus captive populations

The focus of this thesis was captive rather than wild great apes; the prevalence of cardiovascular disease among wild great apes being unknown. The feasibility of carrying out mortality studies in wild populations is limited: sightings are infrequent, animals often do not show clinical signs of disease and fresh diagnostic samples are scarcely available (Boesch 2008). Nonetheless, some such reviews do exist. They cite infectious, and respiratory diseases in particular, to be a major concern for the future sustainability of wild great ape populations,

(Bermejo et al. 2006; IUCN 2012; Leendertz et al. 2006; Nishida et al. 2003; Sakamaki et al. 2009; de Wachter et al. 2003; Walsh et al. 2003). They also show that, especially among chimpanzees, conspecific violence and lethal aggression are also associated with high levels of mortality (Williams et al. 2008). The mortality risk factors to which wild versus zoo-housed great apes are exposed, however, differ greatly. In the zoo environment, infectious disease is controlled, the animals are closely observed for signs of ill health and injury and have access to veterinary intervention where necessary (Wich et al. 2009). In addition, whereas wild great apes must seek out and compete for food, water and shelter and are threatened by the risk of poaching, predation and habitat loss, their zoo-housed counterparts have ready access to these resources and are protected from harm (Williams et al. 2008).

Whether or not cardiovascular disease occurs at all or with the same frequency in wild great apes, or whether it is in fact a disease of captivity, is an important question to answer. A comparison of mortality data would therefore be very informative, as would the detailed post-mortem examination of any hearts collected from wild or even sanctuary-housed animals. Adoption of the methodologies outlined in this thesis for analysis of any such data collected, would allow for meaningful comparison between wild and zoo-housed populations, and for key similarities and differences to be reliably identified.

III Final remarks

The aims and objectives of this thesis were achieved as follows:

1. The ***main causes of mortality*** among the European zoo-housed great ape populations were identified and specifically, the ***relative importance of cardiovascular disease*** among each of the taxa ***was quantified*** *(Chapter 2)*

2. ***The epidemiology of great ape cardiovascular disease was explored*** in greater detailed and ***possible risk factors and areas of interest for further research*** were identified *(Chapter 3)*

3. ***Protocols for the ante-mortem (echocardiographic) and post-mortem investigation of great ape cardiovascular disease were developed and disseminated*** among the wider zoological profession (*Chapter 4*). This has had the effect of:

    a. Encouraging and promoting a pro-active approach to cardiovascular disease screening and research into this topic across European zoological collections

    b. Improving the quality of great ape cardiovascular disease investigation and

    c. Generating a wealth of good quality, consistent and comparable data and samples for future research

4. ***The effects of two different anaesthetic agents on the cardiovascular system*** have been compared, and their suitability for use when using echocardiography to screen for cardiac disease in great apes evaluated *(Chapter 5)*

5. ***The main pathological lesions affecting the great ape cardiovascular system have been identified, describe and characterised*** using a standardised and consistent methodology. They have also been compared with those described in humans and *(Chapter 6)* evidence based inferences about possible aetiologies have been proposed

This thesis represents a significant body of work consisting of several projects, studies and work streams, all of which were focused around improving understanding about great ape mortality and specifically, cardiovascular disease. The ways in which each of these topics and the individual thesis chapters relate to one another are explained by Figure I. Figure II aims to also highlight several avenues for ongoing work (orange) and future research (yellow) in this area.

It is anticipated that the studies carried out will serve as models for similar pan-European multi-centre investigative studies in these, and other, species, in the future. The findings have laid the foundations for the long-term future monitoring and study of great ape mortality and cardiovascular disease. It is hoped that they, and any future work based upon them, will ultimately improve the health and welfare of great apes housed in zoological collection across Europe and Worldwide.

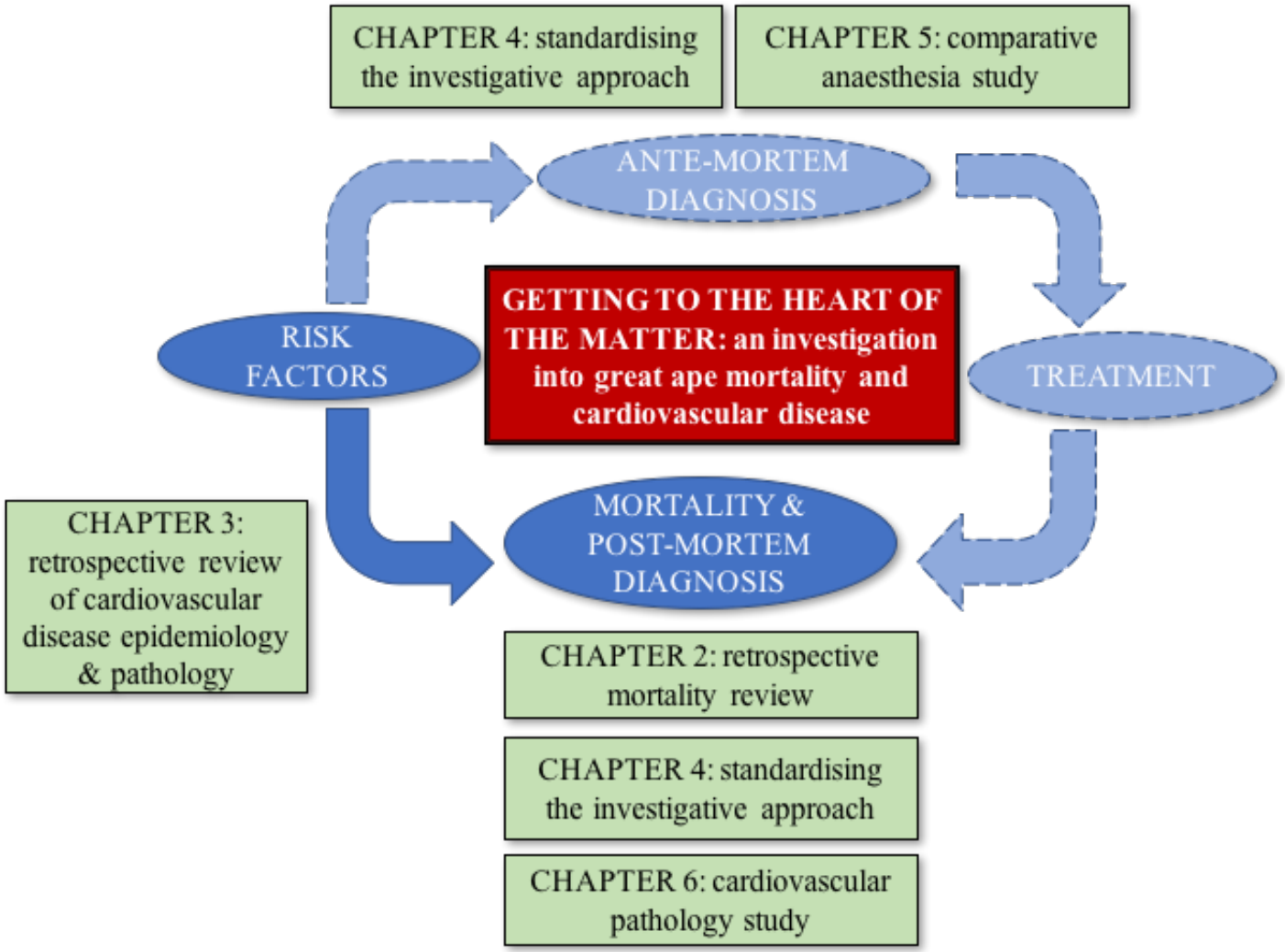


Figure I: Diagram showing the key aspects of great ape cardiovascular disease which are explored as part of this thesis, as well as the relevant chapters within which each topic is covered

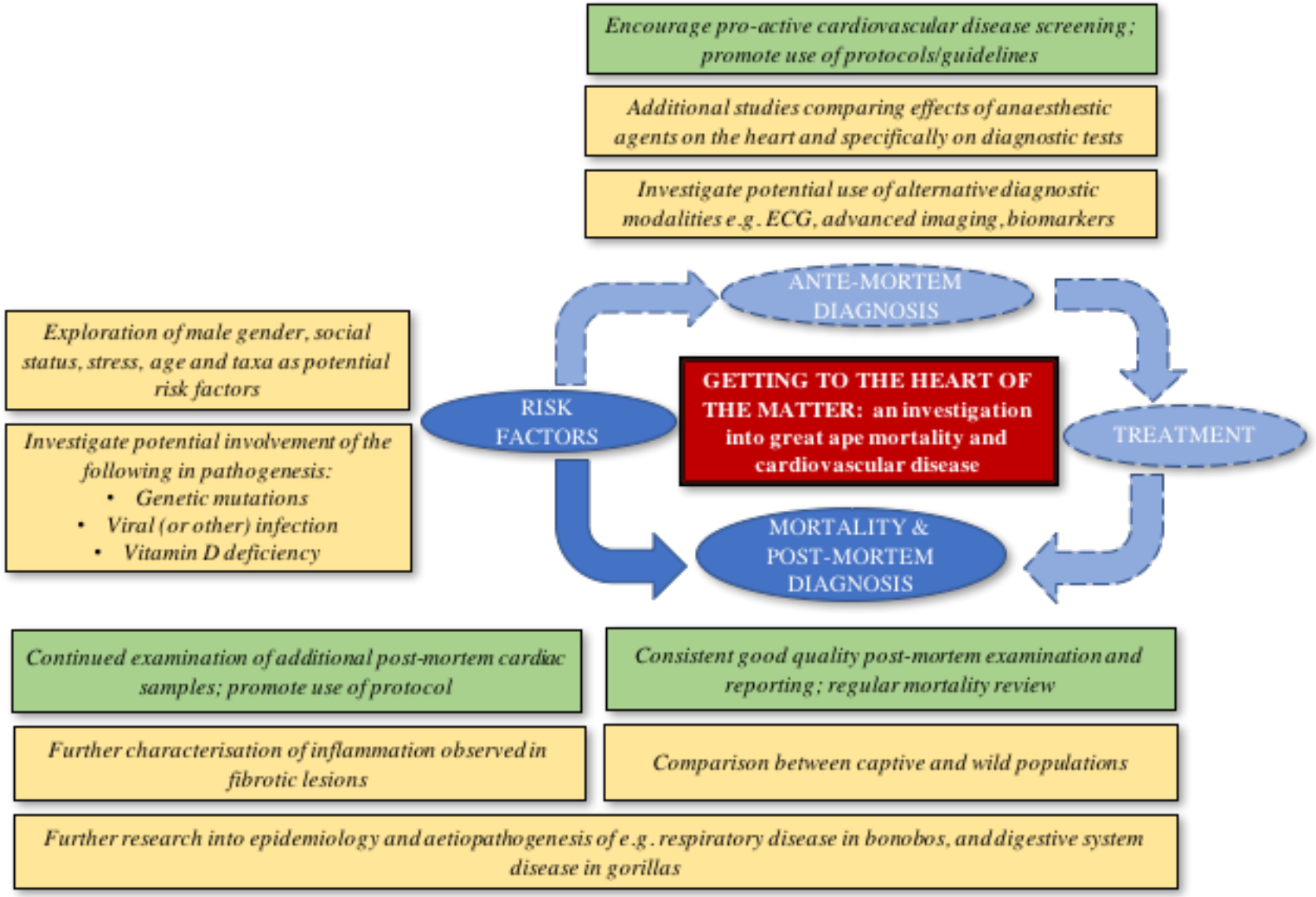


Figure II: Diagram showing several potential avenues for ongoing work (green) and future research (yellow) into great ape mortality and cardiovascular disease

LITERATURE CITED

Aboderin, I. et al., 2002. *Life course perspectives on coronary heart disease, stroke and diabetes: The evidence and implications for policy and research*, World Health Organisation, Geneva.

Adami, C. et al., 2012. Evaluation of effectiveness, safety and reliability of intramuscular medetomidine-ketamine for captive great apes. *The Veterinary Record*, 171(8), p.196.

Alberts, S.C. et al., 2013. Reproductive aging patterns in primates reveal that humans are distinct. *Proceedings of the National Academy of Sciences of the United States of America*, 110, pp.13440–5.

Aldridge, V.J., 2005. A search for probable cause: self-wounding in a zoo chimpanzee (*Pan troglodytes*). Meeting Abstract. *American Journal of Primatology*, 66(Supplement 1), p.82.

Alford, P.L. & Satterfield, W.C., 1995. Paralytic illness resembling inflammatory polyradiculoneuropathy in a chimpanzee. *J Am Vet Med Assoc*., 207(1), pp.83–5.

Allchurch, A.F., 1993. Sudden death and cardiovascular disease in the lowland gorilla. *Dodo*, 29, pp.172–178.

Alvarado, T., 1992. Atypical mycobacterial pneumonia in a western lowland gorilla caused by *Mycobacterium kansasii*. In *AAZPA Regional Conference Proceedings*. p. 629–632.

Amann, R. & Peskar, B.A., 2002. Anti-inflammatory effects of aspirin and sodium salicylate. *European Journal of Pharmacology*, 447(1), pp.1–9.

Anderson, J.L. et al., 2010. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. *American Journal of Cardiology*, 106(7), pp.963–968.

Anderson, K.M. et al., 1991. Cardiovascular disease risk profiles. *American Heart Journal*, 121(1), pp.293–298.

Andréoletti, L. et al., 2009. Viral causes of human myocarditis. *Archives of Cardiovascular Diseases*, 102(6–7), pp.559–568.

Archer, J., 2003. Cardiac biomarkers: a review. *Comparative clinical pathology*, 12, pp.121–128.

Asbun, J. & Villarreal, F.J., 2006. The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. *Journal of the American College of Cardiology*, 47(4), pp.693–700.

Assomull, R.G. et al., 2006. Cardiovascular Magnetic Resonance, Fibrosis, and Prognosis in Dilated Cardiomyopathy. *Journal of the American College of Cardiology*, 48(10), pp.1977–1985.

AZA, 2014. Association of Zoos and Aquariums. Available at: https://www.aza.org/taxon-advisory-groups/ [Accessed January 3, 2017].

AZA Ape TAG, 2010. Chimpanzee (*Pan troglodytes*) Care Manual. Association of Zoos and Aquariums, Silver Spring, MD.

Bain, J., 2007. The many faces of testosterone. *Clinical interventions in aging*, 2(4), pp.567–76.

Baldessari, A. et al., 2013. Fatal myocardial fibrosis in an aged chimpanzee (*Pan troglodytes*). *Pathobiology of Aging & Age Related Diseases*, 3, pp.1–5.

Banish, L.D. et al., 1990. Shigellosis in a zoological collection of primates. *Journal of Zoo and Wildlife Medicine*, 21(3), pp.302–309.

Barratt, H. & Kirwan, M., 2009. Biases: bias in epidemiological studies. *Health Knowledge*. Available at: http://www.healthknowledge.org.uk/public-health-textbook/research-methods/1a-epidemiology/biases [Accessed July 15, 2015].

Barrie, M.T. et al., 1999. Acute lymphocytic leukemia in a six-month-old western lowland gorilla (Gorilla gorilla gorilla). *Journal of Zoo and Wildlife Medicine*, 30, pp.268–272.

Basso, C. et al., 1996. Arrhythmogenic right ventricular cardiomyopathy: dysplasia, dystrophy, or myocarditis? *Circulation*, 94(5), pp.983–991.

Basso, C. et al., 2004. Arrhythmogenic right ventricular cardiomyopathy causing sudden cardiac death in boxer dogs. *Circulation*, 109(9).

Basso, C. et al., 2008. Guidelines for autopsy investigation of sudden cardiac death. *Virchows Archiv*, 452(1), pp.11–18.

Baumgartner, C. et al., 2010. Effects of medetomidine-midazolam-fentanyl IV bolus injections and its reversal by specific antagonists on cardiovascular function in rabbits. *Canadian Journal of Veterinary Research*, 74(4), pp.286–298.

Becker, C., 2015. *International Studbook for Orang-Utan EEP 2013/2014*, Zoo Karlsruhe

Behringer, V. et al., 2013. Measurements of salivary alpha amylase and salivary cortisol in hominoid primates reveal within-species consistency and between-species differences. *PLoS ONE*, 8(4).

Bellisari, A. et al., 2004. A captive infant female gorilla with vitamin D and calcium deficiency: Preliminary description. *American Journal Of Physical Anthropology*, S38, p.61.

Benirschke, K. & Adams, F.D., 1980. Gorilla diseases and causes of death. *Journal of*

Reproduction and Fertility, Suppl 28, pp.139–148.

Bermejo, M. et al., 2006. Ebola outbreak killed 5000 gorillas. *Science (New York, N.Y.)*, 314, p.1564.

Beutin, L. et al., 1996. Animal reservoirs of *Escherichia coli* O157:[H7]. *The Veterinary Record*, 139(3), pp.70–71.

Biernacka, A. & Frangogiannis, N.G., 2011. Aging and cardiac fibrosis. *Aging and Disease*, 2, pp.158–173.

Birkett, L.P. & Newton-Fisher, N.E., 2011. How abnormal is the behaviour of captive, zoo-living chimpanzees? *PLoS ONE*, 6(6), p.e20101.

Blampied, N. le Q. et al., 1992. Surgical removal of an abdominal cyst from a Bornean orang-utan *Pongo pygmaeus pygmaeus*. *Dodo*, 28, pp.165–169.

Bloor, B.C. et al., 1992. Hemodynamic and sedative effects of dexmedetomidine in dog. *The Journal of Pharmacology and Experimental Therapeutics*, 263(2), pp.690–7.

Boesch, C., 2008. Why do chimpanzees die in the forest? The challenges of understanding and controlling for wild ape health. *American Journal of Primatology*, 70(8), pp.722–6.

Bourgeois, S.R. et al., 2007. Combination therapy reduces self-injurious behavior in a chimpanzee (*Pan troglodytes troglodyte*s): a case report. *Journal of Applied Animal Welfare Science*, 10(2), pp.123–40.

Bowker, T.J. et al., 2003. Sudden, unexpected cardiac or unexplained death in England: A national survey. *QJM - Monthly Journal of the Association of Physicians*, 96(4), pp.269–279.

Brack, M. et al., 1997. Suspected dermatophilosis in an adult orangutan (*Pongo pygmaeus*

pygmaeus). *Journal of Zoo and Wildlife Medicine*, 28, pp.336–341.

Bradford, C.M. et al., 2013. Unusual Turner syndrome mosaic with a triple x cell line (47,X/49,XXX) in a western lowland gorilla (*Gorilla gorilla gorilla*). *Journal of Zoo and Wildlife Medicine*, 44(4), pp.1055–8.

Bradshaw, G.A. et al., 2008. Building an inner sanctuary: complex PTSD in chimpanzees. *Journal of Trauma & Dissociation: the official journal of the International Society for the Study of Dissociation (ISSD)*, 9, pp.9–34.

Brainard, B., 2016. Anesthetic concerns for the cardiac examination of the great apes. Available from: https://greatapeheartproject.files.wordpress.com/2016/09/anesthetic-concerns-for-the-cardiac-examination-of-the-great-apes-benjamin-brainard.pdf. [Accessed February 6, 2017]

van den Broeck, J. et al., 2005. Data cleaning: detecting, diagnosing, and editing data abnormalities. *PLoS Medicine*, 2(10), p.e267.

Bronikowski, A.M. et al., 2011. Aging in the natural world: comparative data reveal similar mortality patterns across primates. *Science*, 331(March), pp.1325–1328.

Caldecott, J. & Miles, L., 2005. *World atlas of great apes and their conservation* J. O. Caldecott & L. Miles, eds., Berkeley and Los Angeles, California, USA: University of California Press Ltd.

Cambre, R.C. et al., 1995. Maxillary and ethmoid sinusitis with orbital and intracranial extension in an infant orangutan (*Pongo pygmaeus*). *Journal of Zoo and Wildlife Medicine*, 26(1), pp.144–151.

Canfield, P.J. et al., 1997. Amoebic meningoencephalitis caused by *Balamuthia mandrillaris*

in an orang utan. *Aust Vet J*., 75(2), pp.97–100.

Carlsen, F. & Jongh, T. De, 2015. *European Studbook of Chimpanzee, 2014*, Copenhagen Zoo, Denmark.

Carter, M.L., 1992. Skeletal and dental pathology of wild and captive chimpanzees: clinical and evolutionary implications. *American Journal Of Physical Anthropology*., 90(S16), pp.67–68.

Cassella, C.M. et al., 2012. Prevalence of regurgitation and reingestion in orangutans housed in North American zoos and an examination of factors influencing its occurrence in a single group of Bornean orangutans. *Zoo Biology*, 31(5), pp.609–620.

Celly, C.S. et al., 1997. The comparative hypoxaemic effect of four alpha 2 adrenoceptor agonists (xylazine, romifidine, detomidine and medetomidine) in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, 20(6), pp.464–71.

Centre for Reviews and Dissemination, 2009. *Systematic Reviews CRD's guidance for undertaking reviews in health care*, CRD, University of York.

Cerveny, S. & Sleeman, J., 2014. Great Apes. In *Zoo Animal and Wildlife Immobilization and Anesthesia*. Ames, USA: John Wiley & Sons, Inc., pp. 573–584.

Chai, N. et al., 2009. Treatment of a retroperitoneal abscess by omentalization in an orangutan (*Pongo pygmaeus pygmaeus*). *Journal of Zoo and Wildlife Medicine*, 40(2), pp.350–353.

Chai, N. et al., 2003. A case of osteodystrophy in an orang-utan. In *Erkrankungen der Zootiere: Verhandlungsbericht des 41. Internationalen Symposiums uber die Erkrankungen der Zoo- und Wildtiere, Rome, Italy, 28 May - 1 June, 2003*. pp. 81–86.

Chatfield, J. et al., 2006. Resolution of a hyperprolactinemia in a western lowland gorilla (*Gorilla gorilla gorilla*). *Journal of Zoo and Wildlife Medicine*, 37(4), pp.565–6.

Chatfield, J. et al., 2012. Severe idiopathic hypocalcemia in a juvenile western lowland gorilla, *Gorilla gorilla gorilla*. *Journal of Zoo and Wildlife Medicine*, 43(1), pp.171–3.

Cheitlin, M.D. et al., 2003. ACC/AHA/ASE 2003 guideline update for the clinical application of echocardiography: Summary article. *Journal of the American Society of Echocardiography*, 16(10), pp.1091–1110.

Chiu, E. & Bodley, K., 2010. A psychogeriatrician's home visit to the zoo: a case report. *International Psychogeriatrics*, 22(4), pp.671–673.

CITES, 2013. Convention on International Trade in Endangered Species of Wild Fauna and Flora. *The CITES Appendices*. Available at: http://www.cites.org/eng/app/ [Accessed November 28, 2013].

Clarke, C.J. et al., 1994. Respiratory syncytial virus-associated bronchopneumonia in a young chimpanzee. *Journal of Comparative Pathology*, 110, pp.207–212.

Clutton-Brock, T.H. & Isvaran, K., 2007. Sex differences in ageing in natural populations of vertebrates. *Proceedings of the Royal Society B*, 274(1629), pp.3097–104.

Clyde, V.L., 2016. Bonobo SSP Veterinary Advisor Report. Zoological Society of Milwaukee, Milwaukee, Wisconsin.

van Coillie, S. et al., 2008. The effect of inbreeding on infant mortality in captive bonobos (*Pan paniscus*), determined from DNA analysis of hair samples. *Folia Primatologia*, 79(5), p.321.

Connor-Stroud, F.R. et al., 2014. Extensive vascular mineralization in the brain of a

chimpanzee (*Pan troglodytes*). *Comparative Medicine*, 64(3), pp.224–9.

Cook, R.A. et al., 2010. Choriocarcinoma in a young adult gorilla (*Gorilla gorilla gorilla*): diagnosis, treatment and outcome. In *American Association of Zoo Veterinarians Annual Conference, American Association of Zoo Veterinarians*. pp. 229–230.

Corrado, D. et al., 1997. Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. *Journal of the American College of Cardiology*, 30(6), pp.1512–20.

Cossaboon, C. et al., 2008. Allie's story: the road to recovery: a seven year history of a Sumatran orangutan (*Pongo abelii*) with a neurological disease. *Animal Keepers' Forum*, 35(7), pp.273–280.

Courtenay, J., 1988. Infant mortality in mother-reared captive chimpanzees at Taronga Zoo, Sydney. *Zoo Biology*, 7, pp.61–68.

Cousins, D., 1972. Diseases and injuries in wild and captive gorillas: Gorilla gorilla. *International Zoo Yearbook*, 12(1), pp.211–218.

Cousins, D., 1983. Mortality factors in captive gorillas (Gorilla gorilla). *International Zoo News*, 30, pp.5–17.

D'Agostino, J. et al., 2007. Acute appendicitis in a chimpanzee (*Pan troglodytes*). *Journal of Medical Primatology*, 36(3), pp.119–123.

D'Agostino, R.B. et al., 2013. Cardiovascular disease risk assessment: Insights from Framingham. *Global Heart*, 8(1), pp.11–23.

Davies, M.J., 2000. The cardiomyopathies: an overview. *Heart*, 83(4), p.469 LP-474.

Davies, M.J., 1999. The investigation of sudden cardiac death. *Histopathology*, 34(2), pp.93–

98.

Dec, G.W. et al., 1985. Active myocarditis in the spectrum of acute dilated cardiomyopathies. Clinical features, histologic correlates, and clinical outcome. *The New England Journal of Medicine*, 312(14), pp.885–890.

Delmar, M. & McKenna, W.J., 2010. The cardiac desmosome and arrhythmogenic cardiomyopathies: From gene to disease. *Circulation Research*, 107(6), pp.700–714.

Dennert, R. et al., 2008. Acute viral myocarditis. *European Heart Journal*, 29(17), pp.2073–2082.

Díez, J., 2007. Mechanisms of cardiac fibrosis in hypertension. *Journal of Clinical Hypertension (Greenwich, Conn.)*, 9(7), pp.546–550.

Doane, C.J. et al., 2006. Electrocardiogram abnormalities in captive chimpanzees (*Pan troglodytes)*. *Comparative Medicine*, 56, pp.512–518.

Dodam, J.R. et al., 2004. Cardiopulmonary effects of medetomidine, oxymorphone, or butorphanol in selegiline-treated dogs. *Veterinary Anaesthesia and Analgesia*, 31(2), pp.129–137.

Dugdale, A., 2011. Small animal sedation and premedication. In *Veterinary Anaesthesia: Principles to Practice*. John Wiley & Sons, p. 35.

Dumonceaux, G.A. et al., 1997. Treatment of bilateral nasal polyposis and chronic refractory inhalant allergic rhinitis in a chimpanzee (*Pan troglodytes*). *Journal of Zoo and Wildlife Medicine*, 28(2), pp.215–219.

EAZA, 2013. European Association of Zoos and Aquaria. *Collection planning*. Available at: http://www.eaza.net/activities/cp/Pages/Collection Planning.aspx [Accessed November

28, 2013].

EAZA, 2011. European Association of Zoos and Aquaria. Guidelines for veterinary advisors appointed to EAZA TAGs , EEPs and ESBs.

Ellims, A.H. et al., 2012. Diffuse myocardial fibrosis in hypertrophic cardiomyopathy can be identified by cardiovascular magnetic resonance, and is associated with left ventricular diastolic dysfunction. *Journal of cardiovascular magnetic resonance: official journal of the Society for Cardiovascular Magnetic Resonance*, 14(1), p.76.

Ely, J.J. et al., 2011. Blood pressure reference intervals for healthy adult chimpanzees (*Pan troglodytes*). *Journal of Medical Primatology*, 40, pp.171–180.

Ely, J.J. et al. 2013. Hypertension increases with aging and obesity in chimpanzees (*Pan troglodytes). Zoo Biology*, 32, pp.79–87.

Espinosa-Avilés, D. et al., 2004. Treatment of acute self-aggressive behaviour in a captive gorilla (*Gorilla gorilla gorilla*). *Veterinary Record*, 154(3), pp.401–2.

Feldman, A.M. & McNamara, D., 2000. Myocarditis. *The New England Journal of Medicine*, 343(19), pp.1388–1398.

Ferdowsian, H.R. et al., 2011. Signs of mood and anxiety disorders in chimpanzees. *PLoS ONE*, 6.

Fernandez Bellon, H. et al., 2003. Squamous cell carcinoma of the skin in an albino gorilla (*Gorilla gorilla gorilla*). In *Proceedings of the Institute for Zoo and Wildlife Research. No. 5*. Berlin.

Ferreira, J.P. et al., 2015. Anaesthetic induction and recovery characteristics of a diazepam-ketamine combination compared with propofol in dogs. *Journal of the South African*

Veterinary Association, 86(1), pp.1–7.

Fox, C.S., 2010. Cardiovascular disease risk factors, type 2 diabetes mellitus, and the Framingham Heart Study. *Trends in Cardiovascular Medicine*, 20(3), pp.90–95.

Fujisawa, M. et al., 2014. A case of maxillary sarcoma in a chimpanzee (*Pan troglodytes)*. *Journal of Medical Primatology*, 43(2), pp.111–4.

Furuichi, T. et al., 1998. Population Dynamics of Wild Bonobos (*Pan paniscus*) at Wamba. *International Journal of Primatology*, 19(6), pp.1029–1043.

GAHP, 2016a. The Great Ape Heart Project: resources: anesthesia info. Available at: https://greatapeheartproject.org/resources/anesthesia-info/ [Accessed January 24, 2017].

GAHP, 2016b. The Great Ape Heart Project: projects. Available at: https://greatapeheartproject.org/projects/ [Accessed March 1, 2017].

GAHP, 2012. *The Great Ape Heart Project 2012 white paper: a collaboration to understand heart disease, reduce mortality and improve cardiac health in all four great ape taxa*. Available from: https://greatapeheartproject.files.wordpress.com/2016/07/gahp_whitepaper2012.pdf

Galdikas, B.M.F., 1981. Orangutan reproduction in the wild. In C. E. Graham, ed. *Reproductive Biology of the Great Apes: Comparative and Biomedical Perspectives*. London: Academic Press, pp. 281–299.

Gamble, K.C. et al., 2004. Pathologic review of the chimpanzee (*Pan troglodytes*) 1990-2003. In *Proc. of the American Association of Zoo Veterinarians and American Association of Wildlife Veterinarians and Wildlife Diseases Association Joint Conference*. p. 565–570.

Geer, J.C. et al., 1980. Subendocardial ischemic myocardial lesions associated with severe coronary atherosclerosis. *The American Journal of Pathology*, 98(3), pp.663–80.

Gerlach, T. et al., 2011. Alternative therapeutic options for medical management of epilepsy in apes. *Journal of Zoo and Wildlife Medicine*, 42(2), pp.291–4.

Giovannitti, J.A. et al., 2015. Alpha-2 adrenergic receptor agonists: a review of current clinical applications. *Anesthesia Progress*, 62(1), pp.31–9.

Graphpad, 2016. GraphPad Prism 7 for MacOS X.

Greenberg, M. et al., 1999. Surgical repair of an atrial septal defect in a juvenile Sumatran orangutan (*Pongo pygmaeus sumatraensis*). *Journal of Zoo and Wildlife Medicine*, 30(2), pp.256–261.

Greenwood, A.G. et al., 1995. Renal carcinoma in a chimpanzee (*Pan troglodytes*). *Veterinary Record*, 137(15), pp.380–381.

Grime, M.M. et al., 2016. Delphi Method. In *Wiley StatsRef: Statistics Reference Online*. Chichester, UK: John Wiley & Sons, Ltd, pp. 1–6.

Grindlay, D.J.C., et al., 2012. Searching the veterinary literature: a comparison of the coverage of veterinary journals by nine bibliographic Databases. *Journal of Veterinary Medical Education*, 1, pp.1–9.

Groves, C.P., 2005. Mammal species of the world: a taxonomic and geographic reference. In D. E. Wilson & D. M. Reeder, eds. *Mammal Species of the World: A Taxonomic and Geographic Reference*. Johns Hopkins University Press, pp. 181–184.

Guillot, X. et al., 2010. Vitamin D and inflammation. *Joint Bone Spine*, 77(6), pp.552–557.

Gulati, A. et al., 2013. Association of fibrosis with mortality and sudden cardiac death in

patients with nonischemic dilated cardiomyopathy. *JAMA*, 309(9), pp.896–908.

Gusset, M. et al., 2013. Towards integrated species conservation: editorial. *World Association of Zoos and Aquaria Magazine*, Vol. 14. pp.1

Gustavsson, O.E.A. et al., 1990. An epizootic of whooping cough among chimpanzees in a zoo. *Folia Primatologia*, 55(1), pp.45–50.

Häggström, J. et al., 1992. Chronic valvular disease in the cavalier King Charles spaniel in Sweden. *The Veterinary Record*, 131(24), pp.549–53.

Hare, L.G. et al., 2003. Dietary salicylates. *Journal of Clinical Pathology*, 56(9), pp.649–50.

Harrison, T.M. et al., 2001. *Aeromonas hydrophila* septicemia and resulting encephalopathy in a captive juvenile western lowland gorilla (*Gorilla gorilla gorilla*). In M. M. Baer, Charlotte Kirk; Willette, ed. *Proceedings American Association of Zoo Veterinarians American Association of Wildlife Veterinarians Association of Reptilian and Amphibian Veterinarians National Association of Zoo and Wildlife Veterinarians Joint Conference Orlando, Florida September 18*. American Association of Zoo Veterinarians, pp. 366–370.

Hayashi, Y. et al., 1991. Dexmedetomidine prevents epinephrine-induced arrhythmias through stimulation of central alpha 2 adrenoceptors in halothane-anesthetized dogs. *Anesthesiology*, 75(1), pp.113–117.

Hayman, D.T. et al., 2010. Successful treatment of acute systemic anaphylaxis in a western lowland gorilla (*Gorilla gorilla gorilla*). *Journal of Zoo and Wildlife Medicine*, 41(3), pp.522–5.

Herrin, K. V, et al., 2005. Posaconazole therapy for systemic coccidioidomycosis in a

chimpanzee (*Pan troglodytes)*: a case report. *Mycoses*, 48(6), pp.447–52.

Hewitt, S., 2005. Preliminary findings of the prevalence of diseases in captive western lowland gorillas (*Gorilla gorilla gorilla*) in the United Kingdom and Ireland (1896-2005). In *Proceedings of the 7th Annual Symposium on Zoo Research, Twycross Zoo, Warwickshire, UK, 7-8th July 2005*. pp. 222–231.

Heymans, S. et al., 2015. Searching for new mechanisms of myocardial fibrosis with diagnostic and/or therapeutic potential. *European Journal of Heart Failure*, 6(17), pp.764–771.

Higgs, G. A et al., 1987. Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. *Proceedings of the National Academy of Sciences of the United States of America*, 84(5), pp.1417–1420.

Hill, K. et al., 2001. Mortality rates among wild chimpanzees. *Journal of Human Evolution*, 40(5), pp.437–50.

Hill, S.P., 2009. Do gorillas regurgitate potentially-injurious stomach acid during 'regurgitation and reingestion? *Animal Welfare*, 18, pp.123–127.

Ho, C.Y. et al., 2010. Myocardial fibrosis as an early manifestation of hypertrophic cardiomyopathy. *The New England Journal of Medicine*, 363(6), pp.552–63.

Ho, K.K.L. et al., 1993. The epidemiology of heart failure: the Framingham Study. *Journal of the American College of Cardiology*, 22(4 SUPPL. 1).

Hobel, C.J. & Arora, C.P., 2010. Cardiovascular disease risk factors for women. A life course-events perspective. *Biopolymers and Cell*, 26, pp.87–96.

Hoffman, K. et al., 2007. Diagnosis and treatment of ocular coccidioidomycosis in a female

captive chimpanzee (*Pan troglodytes)*: a case study. *Annals of the New York Academy of Sciences*, 1111, pp.404–10.

Hohmann, G. et al., 2009. The relationship between socio-sexual behavior and salivary cortisol in bonobos: tests of the tension regulation hypothesis. *American Journal of Primatology*, 71(3), pp.223–232.

Holick, M.F., 2004. Vitamin D: Importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. In *American Journal of Clinical Nutrition*. pp. 362–371.

Hooper, J., 2011. Primary hyperparathyroidism in a western lowland gorilla. In V. Roberts, ed. *British Veterinary Zoological Society Proceedings of the Spring Meeting: Mammalian Medicine and Surgery*. Ashford, p. 23.

Hunter, R. & Agnew, A., 2011. A differential diagnosis of diffuse idiopathic skeletal hyperostosis (DISH) in a *Gorilla gorilla gorilla* specimen. *American Journal of Anthropology: 80th Annual Meeting of the American-Association-of-Physical-Anthropologists*, 144(S52), p.172.

Ihms, E.A. et al., 2006. Fatal *Streptococcus anginosus*-associated pneumonia in a captive Sumatran orangutan (*Pongo abelii*). *Zoo Biology*, 25(2), pp.101–115.

Institute of Medicine, 2010. *Promoting cardiovascular health in the developing world: a critical challenge to achieve global health*. The National Academies Press. pp. 482. Available from: http://www.nap.edu/catalog/12815.html%5Cnhttp://books.google.com/books?hl=en&lr=&id=uNEoYaJ_NWwC&oi=fnd&pg=PT1&dq=Promoting+Cardiovascular+Health+in+the+Developing+World:+A+Critical+Challenge+to+Achieve+Global+Health&ots=iobfgg73KJ

International Species Information System (ISIS), 2013. International Species Information System: Zoological Information System (ZIMS) database.

International Union for Conservation of Nature, 2013. IUCN. *The IUCN Red List of Threatened Species. Version 2013*.

Isidoro, M., et al., 2013. Haemorrhagic-necrotizing colitis associated with *campylobacter jejuni* and *balantidium coli* in a zoo gorilla. *Journal of Comparative Pathology*, 148(1), p.87.

IUCN, 2012. *Bonobo (Pan paniscus) Conservation Strategy 2012–2022*, IUCN/SSC Primate Specialist Group & Institut Congolais pour la Conservation de la Nature.

IUCN, 2016. The IUCN Red List of Threatened Species. Version 2016-3. Available at: http://www.iucnredlist.org [Accessed January 3, 2017].

Janssen, D.L.& & Bush, R.M., 1990. Review of the medical literature of great apes in the 1980s. *Zoo Biology*, 9, pp.123–134.

Jean, S.M. et al., 2012. Cerebrovascular accident (stroke) in captive, group-housed, female chimpanzees. *Comparative Medicine*, 62(4), pp.322–9.

Jellis, C. et al., 2010. Assessment of nonischemic myocardial fibrosis. *Journal of the American College of Cardiology*, 56(2), pp.89–97.

John, B.T. et al., 2004. Global remodeling of the ventricular interstitium in idiopathic myocardial fibrosis and sudden cardiac death 1. *Heart Rhythm*, 1(2), pp.141–149.

Jones, P. et al., 2011. Encephalomyocarditis virus mortality in semi-wild bonobos (*Pan panicus*). *Journal of Medical Primatology*, 40, pp.157–163.

de Jong, S. et al., 2011. Biomarkers of myocardial fibrosis. *Journal of Cardiovascular*

Pharmacology, 57, pp.522–535.

Jousilahti, P. et al., 1999. Sex, age, cardiovascular risk factors, and coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and women in Finland. *Circulation*, 99(9), pp.1165–72.

Junge, R.E. et al., 2000. Management and prevention of vitamin D deficiency rickets in captive-born juvenile chimpanzees (*Pan troglodytes*). *Journal of Zoo and Wildlife Medicine*, 31(3), pp.361–9.

Kajstura, J. et al., 1996. Necrotic and apoptotic myocyte cell death in the aging heart of Fischer 344 rats. *The American Journal of Physiology*, 271(3 Pt 2), pp.H1215-28.

Kallio, A. et al., 1990. Acute hemodynamic effects of medetomidine and clonidine in healthy volunteers: a noninvasive echocardiographic study. *Journal of Cardiovascular Pharmacology*, 16(1), pp.28–33.

Kania, G. et al., 2009. Mechanisms of cardiac fibrosis in inflammatory heart disease. *Trends in Cardiovascular Medicine*, 19(8), pp.247–252.

Kannel, W.B. & McGee, D.L., 1979. Diabetes and cardiovascular risk factors: the Framingham study. *Circulation*, 59(1), pp.8–13.

Karesh, W.B. et al., 2012. Clinical challenge case 1: aspiration pneumonia and abscess in a gorilla. *Primates*, 53(3), pp.221–226.

Kästner, S.B., 2006. A2-agonists in sheep: a review. *Veterinary Anaesthesia and Analgesia*, 33(2), pp.79–96.

Kawai, C., 1999. From myocarditis to cardiomyopathy: mechanisms of inflammation and cell death: learning from the past for the future. *Circulation*, 99(8), pp.1091–100.

Kearney, M.T. et al., 2001. Viral myocarditis and dilated cardiomyopathy: mechanisms, manifestations, and management. *Postgrad Med J*, 77, pp.4–10.

Kennedy, M.J. & Smith, L.J., 2015. A comparison of cardiopulmonary function, recovery quality, and total dosages required for induction and total intravenous anesthesia with propofol versus a propofol-ketamine combination in healthy Beagle dogs. *Veterinary Anaesthesia and Analgesia*, 42(4), pp.350–359.

Kenny, D.E. et al., 1994. Aortic dissection - an important cardiovascular-disease in captive gorillas (*Gorilla gorilla gorilla*). *Journal of Zoo and Wildlife Medicine*, 25, pp.561–568.

Kenny, D.E. et al., 2003. Negative-pressure pulmonary edema complicated by acute respiratory distress syndrome in an orangutan (*Pongo pygmaeus abelii*). *Journal of Zoo and Wildlife Medicine*, 34, pp.394–399.

Kerr, C.L., et al, 1996. A comparison of romifidine and xylazine when used with diazepam/ketamine for short duration anesthesia in the horse. *Canadian Veterinary Journal*, 37(10), pp.601–609.

Khan, Z.P. et al. 1999. Alpha-2 and imidazoline receptor agonists: their pharmacology and therapeutic role. *Anaesthesia*, 54(2), pp.146–165.

Kik, M.J. et al., 2005. Herpes simplex infection in a juvenile orangutan (*Pongo pygmaeus pygmaeus*). *Journal of Zoo and Wildlife Medicine*, 36(1), pp.131–4.

Kittleson, M.D. et al., 1999. Familial Hypertrophic Cardiomyopathy in Maine Coon Cats. *Circulation*, 99(24).

Kittleson, M.D. & Kienle, R.D., 1998. *Small Animal Cardiovascular Medicine*, Mosby.

Kitzman, D.W. et al., 1988. Age-related changes in normal human hearts during the first 10

decades of life. Part II (Maturity): A quantitative anatomic study of 765 specimens from subjects 20 to 99 years old. *Mayo Clinic Proceedings*, 63(2), pp.137–46.

Kitzman, D.W. & Edwards, W.D., 1990. Age-related changes in the anatomy of the normal human heart. *Journal of Gerontology*, 45(2), pp.M33-9.

Klopfleisch, R. et al., 2007. Nevus lipomatosus cutaneus superficialis (Hoffmann-Zurhelle) in a Chimpanzee (*Pan troglodytes*). *Journal of Medical Primatology*, 36, pp.57–60.

Kuh, D. & Shlomo, Y. Ben, 2004. *A Life Course Approach to Chronic Disease Epidemiology*. *Chapter 1: Introduction* 2nd ed. D. Kuh & Y. Ben Shlomo, eds., Oxford University Press.

Kumar, V. et al. 2013. Robbins Basic Pathology. 9th edition. Elsevier/Saunders. Philadelphia.

Lammey, M.L., Baskin, G.B., et al., 2008. Interstitial myocardial fibrosis in a captive chimpanzee (*Pan troglodytes*) population. *Comparative Medicine*, 58, pp.389–394.

Lammey, M.L., Lee, D.R., et al., 2008. Sudden cardiac death in 13 captive chimpanzees (*Pan troglodytes*). *Journal of Medical Primatology*, 37 Suppl 1(13), pp.39–43.

Lammey, M.L. et al., 2011. Use of an implantable loop recorder in the investigation of arrhythmias in adult captive chimpanzees (*Pan troglodytes*). *Comparative medicine*, 61, pp.71–75.

Lankester, F. et al., 2008. Fatal ulcerative colitis in a western lowland gorilla (*Gorilla gorilla gorilla*). *Journal of Medical Primatology*, 37(6), pp.287–302.

Lawrence, C., et al. 1996. The effect of dexmedetomidine on nutrient organ blood flow. *Anesthesia & Analgesia*, pp.1160–1165.

Lawson, B., et al. B.M.F., 2006. Airsacculitis in fourteen juvenile southern Bornean

orangutans (*Pongo pygmaeus wurmbii*). *Journal of Medical Primatology*, 35, pp.149–154.

Lear, T.L. et al., 2001. Trisomy 17 in a bonobo (*Pan paniscus*) and deletion of 3q in a lowland gorilla (*Gorilla gorilla gorilla*): comparison with human trisomy 18 and human deletion 4q syndrome. *Cytogenetics and Cell Genetics*, 95(3–4), pp.228–33.

Leendertz, F.H. et al., 2006. Anthrax in western and central African great apes. *American Journal of Primatology*, 68, pp.928–933.

Lewis, I., et al., 2001. Management of a chronically ill orangutan: communication and cooperation are the keys. In *Brookfield Zoo [Ed.]. The apes: challenges for the 21st century. Conference proceedings. Chicago Zoological Society, Brookfield*. pp. 186–189.

Lewis, J.C.M., 1993. Medetomidine-ketamine anaesthesia in the chimpanzee (*Pan Troglodytes*). *Veterinary Anaesthesia and Analgesia*, 20, pp.18–20.

Lintzenich, B. & Ward, A.M., 2001. Weight management strategies in apes. In *Brookfield Zoo [Ed.]. The apes: challenges for the 21st century. Conference proceedings. Chicago Zoological Society, Brookfield. 2001: i-viii, 1-376*. Chicago Zoological Society, Brookfield, pp. 175–176.

López, B. et al., 2008. Myocardial fibrosis in chronic kidney disease: potential benefits of torasemide. *Kidney International*, 74(111), pp.S19–S23.

López, B. et al., 2015. Circulating biomarkers of myocardial fibrosis. *Journal of the American College of Cardiology*, 65(22).

Lowenstine, L.. et al., 2008. Preliminary results of a survey of United States and Canadian orangutan mortalities in the North American SSP population from 1980-March 2008. In

American Association of Zoo Veterinarians. p. 40.

Lucas, S.B., 2011. Derivation of new reference tables for human heart weights in light of increasing body mass index. *Journal of Clinical Pathology*, 64(4), pp.279–280.

Lukas, K.E. et al., 1999. Removing milk from captive gorilla diets: the impact on regurgitation and reingestion (R/R) and other behaviors. *Zoo Biology*, 18(6), pp.515–528.

Lung, N. et al., 2004. Surgical and medical management of necrotizing fasciitis in the throat sac region of an adult male sumatran orangutan (*Pongo pygmaeus abelii*). In *Proceedings of the American Association of Zoo Veterinarians*. p. 195.

Macri, S.C. et al., 2012. Immunophenotypic alterations in resident immune cells and myocardial fibrosis in the aging rhesus macaque (*Macaca mulatta*) Heart. *Toxicologic Pathology*, 40(4), pp.637–646.

Maddison, J. et al., 2008. Small animal clinical pharmacology: chapter 5: anaesthetic agents. In *Small Animal Clinical Pharmacology*.

Mahrholdt, H. et al., 2006. Presentation, patterns of myocardial damage, and clinical course of viral myocarditis. *Circulation*, 114(15), pp.1581–1590.

Mann, D.L. et al., 2014. *Braunwald's Heart Disease: a textbook of cardiovascular medicine* 10th ed., Philadelphia: Elsevier Health Sciences.

Maron, B.J. & Pelliccia, A., 2006. The heart of trained athletes: cardiac remodeling and the risks of sports, including sudden death. *Circulation*, 114(15), pp.1633–1644.

Márquez, M. et al., 2008. Neuropathologic findings in an aged albino gorilla. *Veterinary Pathology*, 45(4), pp.531–7.

Mätz-Rensing, K. et al., 2011. Fatal *Balamuthia mandrillaris* infection in a gorilla - first case of balamuthiasis in Germany. *Journal of Medical Primatology*, 40(6), pp.437–40.

Mbaya, A.W. & Nwosu, C.O., 2005. Outbreak of clinical amoebosis among captive chimpanzees (*Pan troglodytes*) at the Sanda Kyarima park, Maiduguri, Nigeria. *Nigerian Veterinary Journal*, 27(1), pp.68–73.

Mckenna, W.J. et al., 1994. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *British Heart Journal*. 71, pp.215–218.

McLachlan, S.M. et al.., 2011. Review and hypothesis: does Graves' disease develop in non-human great apes? *Thyroid*, 21(12), pp.1359–66.

McMillan, C.O. et al., 1992. Premedication of children with oral midazolam. *Canadian Journal of Anaesthesia*, 39(6), pp.545–550.

McMullen, J.R. & Jennings, G.L., 2007. Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure. *Clinical and Experimental Pharmacology and Physiology*, 34(4), pp.255–262.

Meehan, T.P. et al., 1995. Surgical removal of an intracranial tumor in a western lowland gorilla. In R. E. Junge, ed. *Proceedings of a Joint Conference American Association of Zoo Veterinarians, Wildlife Disease Association, and American Association of Wildlife Veterinarians*. East Lansing, Michigan, USA: AAZV, AAWV, and WDA {a}, 810 East 10th Street, Lawrence, Kansas 66044, USA, pp. 255–256.

Meehan, T.P. & Lowenstine, L.J., 1994. Causes of mortality in captive lowland gorillas: a survey of the SSP population. In *Proc American Association Zoo Veterinarians*. p. 216–218.

Mesle, F., 1999. Classifying causes of death according to an aetiological axis. *Population Studies*, 53(1), pp.97–105.

Meurs, K.M. et al., 2014. Natural history of arrhythmogenic right ventricular cardiomyopathy in the boxer dog: a prospective study. *Journal of Veterinary Internal Medicine*, 28(4), pp.1214–1220.

Mewton, N. et al., 2011. Assessment of myocardial fibrosis with cardiovascular magnetic resonance. *Journal of the American College of Cardiology*, 57(8), pp.891–903.

Microsoft, 2010. Microsoft Excel. Microsoft Office. Version 14.0.7147.5001. Redmond, Washington: Microsoft, 2010. Computer Software.

Miller, C.L. et al., 1999. Chronic hypertension with subsequent congestive heart failure in a western lowland gorilla (*Gorilla gorilla gorilla*). *Journal of Zoo and Wildlife Medicine*, 30, pp.262–267.

Minter, L.J. et al., 2012. Reye's or Reye's-like syndrome in western lowland gorilla (*Gorilla gorilla gorilla*). *Journal of Medical Primatology*, 41, pp.329–331.

Miyabe-Nishiwaki, T. et al., 2010. Tetraparesis resembling acute transverse myelitis in a captive chimpanzee (*Pan troglodytes*): long-term care and recovery. *Journal of Medical Primatology*, 39(5), pp.336–46.

Miyagi, J. et al., 1999. Coxsackievirus B4 myocarditis in an orangutan. *Veterinary Pathology*, 36(5), pp.452–456.

Moyna, B.K., 2012. Anaesthesia for patients with cardiac disease undergoing non-cardiac surgery. *Anaesthesia & Intensive Care Medicine*, 13(10), pp.519–523.

Mundy, N.I. et al., 1998. Protein deficiency in a colony of western lowland gorillas (*Gorilla*

gorilla gorilla). *Journal of Zoo and Wildlife Medicine*, 29(3), pp.261–8.

Munson, L. & Montali, R.J., 1990. Pathology and diseases of great apes at the National Zoological Park. *Zoo Biology*, 9, pp.99–105.

Murphy, H.W. et al., 2011. Echocardiographic parameters of captive western lowland gorillas (*Gorilla gorilla gorilla*). *Journal of Zoo and Wildlife Medicine*, 42, pp.572–9.

Murray, S. et al., 2000. Diverticulitis with rupture and fatal peritonitis in a Sumatran orangutan (*Pongo pygmaeus*). *Comparative Medicine*, 50(4), pp.452–4.

Mylniczenko, N.D., 2003. A preliminary report on intra-abdominal abscesses in captive western lowland gorillas (*Gorilla gorilla gorilla*). In C. K. Baer, ed. *American Association of Zoo Veterinarians Annual Conference, Minneapolis, Minnesota, October 4-10, 2003. Proceedings. American Association of Zoo Veterinarians, place of publication not given*. American Association of Zoo Veterinarians, pp. 62–66.

Naguib, M. & Samarkandi, A.H., 2000. The comparative dose-response effects of melatonin and midazolam for premedication of adult patients: a double-blinded, placebo-controlled study. *Anesthesia & Analgesia*, 91(2), pp.473–479.

Napier, J.E. et al., 2013. Evaluating echocardiogram and indirect blood pressure results in male western lowland gorillas (*Gorilla gorilla gorilla*) during three phases of an anesthetic protocol. *Journal of Zoo and Wildlife Medicine*, 44, pp.875–881.

Naples, L., et al., 2010. Comparison of the anesthetic effects of oral transmucosal versus injectable medetomidine in combination with tiletamine-zolazepam for immobilization of chimpanzees (*Pan troglodytes*). *Journal of Zoo and Wildlife Medicine*, 41(1), pp.50–62.

Nascimento, J. dos S. et al., 2007. Sedative and cardiovascular effects of midazolam and diazepam alone or combined with clonidine in patients undergoing hemodynamic studies for suspected coronary artery disease. *Arquivos Brasileiros de Cardiologia*, 89(6), pp.403–8.

National heart lung and blood institute, 2016. Types of cardiomyopathy. *Health Information for the public: lealth topics: cardiomyopathy*. Available at: https://www.nhlbi.nih.gov/health/health-topics/topics/cm/types [Accessed March 3, 2017].

Nielsen, S.C.A. et al., 2012. Probable transmission of coxsackie B3 virus from human to chimpanzee, Denmark. *Emerging Infectious Diseases*, 18, pp.1163–1165.

Nishida, T. et al., 2003. Demography, female life history, and reproductive profiles among the chimpanzees of Mahale. *American Journal of Primatology*, 59(3), pp.99–121.

Nunamaker, E. A. et al., 2012. Chronic diseases in captive geriatric female Chimpanzees (*Pan troglodytes*). *Comparative Medicine*, 62, pp.131–6.

O'Hanlon, R. et al., 2010. Prognostic significance of myocardial fibrosis in hypertrophic cardiomyopathy. *J Am Coll Cardiol*, 56, pp.867–874.

Olivetti, G. et al., 1991. Cardiomyopathy of the aging human heart: myocyte loss and reactive cellular hypertrophy. *Circulation Research*, 68(6), pp.1560–1568.

Oxford University Press, 2017. English Oxford Dictionary: Online. Available at: https://en.oxforddictionaries.com/definition/pathology [Accessed March 1, 2017].

Paixão, T.A. et al., 2014. Hemorrhagic colitis associated with *Salmonella enterica* serotype Infantis infection in a captive western lowland gorilla (*Gorilla gorilla gorilla*) in Brazil.

Journal of Medical Primatology, 43(2), pp.118–121.

Paixão, T.A. et al., 2014. Pathological findings in a captive senile western lowland gorilla (*Gorilla gorilla gorilla*) with chronic renal failure and septic polyarthritis. *Brazilian Journal of Veterinary Pathology*, 7(February 2012), pp.29–34.

Pakkanen, S.A. et al., 2015. Detomidine and the combination of detomidine and MK-467, a peripheral alpha-2 adrenoceptor antagonist, as premedication in horses anaesthetized with isoflurane. *Veterinary Anaesthesia and Analgesia*, 42(5), pp.527–536.

Pazzaglia, G. et al., 1994. Persistent, recurring diarrhea in a colony of orangutans (*Pongo pygmaeus*) caused by multiple strains of Campylobacter spp. *Acta Tropica*, 57(1), pp.1–10.

Pereboom, J.J.M. et al., 2015. *International Studbook for Bonobo (Pan paniscus): Schwarz 1929*,

Pérez-López, F.R. et al., 2010. Gender differences in cardiovascular disease: hormonal and biochemical influences. *Reproductive sciences (Thousand Oaks, Calif.)*, 17, pp.511–531.

Peters, S., 2006. Advances in the diagnostic management of arrhythmogenic right ventricular dysplasia–cardiomyopathy. *International Journal of Cardiology*, 113(1), pp.4–11.

Pilz, S. et al., 2010. Vitamin D deficiency and myocardial diseases. *Molecular Nutrition and Food Research*, 54(8), pp.1103–1113.

Pinamonti, B. et al., 2014. Arrhythmogenic right ventricular cardiomyopathy: From genetics to diagnostic and therapeutic challenges. *World journal of cardiology*, 6(12), pp.1234–44.

Pinedo, M. et al., 2010. Inter- and intra-observer variability in the echocardiographic evaluation of right ventricular function. *Revista Espanola de Cardiologia*, 63(7), pp.802–9.

Pollock, P. et al., 2008. Repeat laparotomy for the treatment of septic peritonitis in a Bornean orangutan (*Pongo pygmaeus pygmaeus*). *Journal of Zoo and Wildlife Medicine*, 39(3), pp.476–479.

Porter, B. et al., 2004. A case report of hepatocellular carcinoma and focal nodular hyperplasia with a myelolipoma in two chimpanzees and a review of spontaneous hepatobiliary tumors in non-human primates. *Journal of Medical Primatology*, 33(1), pp.38–47.

PRISMA Group et al., 2009. *Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. The PRISMA Group. Available from: http://www.prisma-statement.org*

Prosen, H. & Bell, B., 2001. A psychiatrist consulting at the zoo (The therapy of Brian the bonobo). In *Brookfield Zoo [Ed.]. The apes: challenges for the 21st century. Conference proceedings. Chicago Zoological Society, Brookfield. 2001: i-viii, 1-376*. pp. 161–164.

Quesenberry, K.E. et al., 2004. Chapter 33 – Anesthesia, analgesia, and sedation of small mammals. In *Ferrets, rabbits, and rodents*. pp. 356–369.

Rand, J.S. et al., 1996. Echocardiographic evaluation of the effects of medetomidine and xylazine in dogs. *Australian Veterinary Journal*, 73(2), pp.41–44.

Raphael, B.L. et al., 1995. Reactive arthritis subsequent to *Shigella flexneri* enteritis in two juvenile lowland gorillas (*Gorilla gorilla gorilla*). *Journal of Zoo and Wildlife Medicine*, 26(1), pp.132–138.

Reddacliff, L.A. et al., 1997. Encephalomyocarditis virus infections in an Australian zoo. *Journal of Zoo and Wildlife Medicine*, 28, pp.153–157.

Reinartz, G.. et al., 2002. *Bonobo (Pan paniscus) Master Plan 2002: Recommendations for the Global Captive Population. III: Demographic Characteristics: Analyses and Results*. Zoological Society of Milwaukee, Milwaukee, Wisconsin.

Richardson, P. et al., 1996. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies. *Circulation*, 93(5), p.841 LP-842.

Rideout, B.A. et al., 1997. Fatal infections with *Balamuthia mandrillaris* (a free-living amoeba) in gorillas and other Old World primates. *Veterinary Pathology*, 34, pp.15–22.

Rietschel, W., 2012. Air sacculitis in bonobo (*Pan paniscus*) and chimpanzee (*Pan troglodytes*) - anatomy, pathology and therapy. In *Proceedings of the International Conference on Diseases of Zoo and Wild Animals 2012, May 16th - 19th, 2012, Bussolengo/Italy*. pp. 112–115.

Rinelli, S. et al., 2012. Circulating salicylic acid and metabolic and inflammatory responses after fruit ingestion. *Plant Foods for Human Nutrition*, 67(1), pp.100–104.

Rizzo, F. et al., 2007. Thrombocytosis in cats: a retrospective study of 51 cases (2000-2005). *Journal of Feline Medicine and Surgery*, 9, pp.319–325.

Robinson, N. et al., 2015. Investigating common clinical presentations in first opinion small animal consultations using direct observation. The *Veterinary Record*, 176(18).

Rolff, J., 2002. Bateman's principle and immunity. *Proc Biol Sci*, 269(1493), pp.867–872.

Romagnoli, N. et al., 2016. Non-invasive evaluation of the haemodynamic effects of high-

dose medetomidine in healthy cats for semen collection. *Journal of Feline Medicine and Surgery*, 18(4), pp.337–343.

Rosano, G.M. & Panina, G., 1999. Oestrogens and the heart. *Therapie*, 54(3), pp.381–5.

Rowe, N., 1996. *The pictorial guide to the living primates*, Pogonias Press Inc. Charlestown, Rhode Island.

Sabatini, C.F. et al., 2013. Effects of injectable anesthetic combinations on left ventricular function and cardiac morphology in Sprague-Dawley rats. *Journal of the American Association for Laboratory Animal Science : JAALAS*, 52(1), pp.34–43.

Sakamaki, T., Mulavwa, M. & Furuichi, T., 2009. Flu-like epidemics in wild bonobos (*Pan paniscus*) at Wamba, the Luo Scientific Reserve, Democratic Republic of Congo. *Pan Africa News*, 16(1), pp.1–4.

Sakulwira, K. et al., 2004. Orangutan herpesvirus. *Journal of Medical Primatology*, 33, pp.25–29.

Sangaralingham, S.J. et al., 2011. The aging heart, myocardial fibrosis, and its relationship to circulating C-type natriuretic peptide. *Hypertension (Dallas, Tex. : 1979)*, 57(2), pp.201–7.

Sapolsky, R.M., 2005. Review: the Influence of social hierarchy on primate health. *Science*, 308(5722), pp.648–652.

Saponaro, V. et al., 2013. Echocardiographic evaluation of the cardiovascular effects of medetomidine, acepromazine and their combination in healthy dogs. *Research in Veterinary Science*, 95(2), pp.687–692.

Sasseville, V.G. et al., 2013. Meeting report: emerging respiratory viral infections and

nonhuman primate case reports. *Veterinary Pathology*, 50(6), pp.1145–53.

Saturday, G.A. et al., 2005. KIT-positive gastrointestinal stromal tumor in a 22-year-old male chimpanzee (*Pan troglodytes*). *Veterinary Pathology*, 42, pp.362–365.

Schalla, S. et al., 2010. Replacement and reactive myocardial fibrosis in idiopathic dilated cardiomyopathy: comparison of magnetic resonance imaging with right ventricular biopsy. *European Journal of Heart Failure*, 12(3), pp.227–231.

Schauer, G. et al., 1994. Malignant rhabdoid tumor in the gastric wall of an aged orangutan (*Pongo pygmaeus*). *Veterinary Pathology*, 31, pp.510–517.

Schmidt, R.E., 1975. Systemic pathology of chimpanzees. *Journal of Medical Primatology*, 7, pp.274–318.

Schubert, G. et al., 2013. Co–residence between males and their mothers and grandmothers is more frequent in bonobos than chimpanzees. R. Stanyon, ed. *PLoS ONE*, 8(12), p.e83870.

Schulman, F.Y. et al., 1995. Fibrosing cardiomyopathy in captive western lowland gorillas (*Gorilla gorilla* go*rilla*) in the United-States - a retrospective study. *Journal of Zoo and Wildlife Medicine*, 26, pp.43–51.

Scott, N.A. et al., 1995. In-vivo diagnosis of coronary-artery disease in a western lowland gorilla (*Gorilla gorilla gorilla*). *Journal of Zoo and Wildlife Medicine*, 26, pp.139–143.

Sen-Chowdhry, S. et al., 2007. Clinical and genetic characterization of families with arrhythmogenic right ventricular dysplasia/cardiomyopathy provides novel insights into patterns of disease expression. *Circulation*, 115(13), pp.1710–1720.

Sen-Chowdhry, S. et al., 2008. Left-dominant arrhythmogenic cardiomyopathy: an under-

recognized clinical entity. *Journal of the American College of Cardiology*, 52(25), pp.2175–2187.

Senior, J.M., 2013. Morbidity, mortality, and risk of general anesthesia in horses. *The Veterinary Clinics of North America. Equine practice*, 29, pp.1–18.

Shave, R. et al., 2013. Echocardiographic assessment of cardiac structure and function in great apes: a practical guide. *International Zoo Yearbook*, 48(1), pp. 218-233

Sheppard, M. & Davies, M., 1998. Cardiac hypertrophy, myocarditis and cardiomyopathy. In *Practical Cardiovascular Pathology*. London: Arnold Hodder, pp. 103–148.

Sheppard, M.N., 2012. Approach to the cardiac autopsy. *Journal of Clinical Pathology*, 65(6), pp.484–95.

Sheta, S.A. & Alsarheed, M., 2009. Oral midazolam premedication for children undergoing general anaesthesia for dental care. *International Journal of Pediatrics*, 2009, p.274380.

Shin, N.S. et al., 1995. *Mycobacterium tuberculosis* infection in an orangutan (*Pongo pygmaeus*). *Journal of Veterinary Medical Science*, 57, pp.951–953.

Silva, A.E. et al., 2006. Uterine leiomyoma in chimpanzee (*Pan troglodytes*). *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*, 58(1), pp.129–132.

Simpson, S. et al., 2015. Genetics of human and canine dilated cardiomyopathy. *International Journal of Genomics*, 2015, p.204823.

Sinclair, M.D., 2003. A review of the physiological effects of alpha2-agonists related to the clinical use of medetomidine in small animal practice. *The Canadian Veterinary Journal = La Revue Vétérinaire Canadienne*, 44(11), pp.885–97.

Singh, K.P. et al., 1994. Chronic pathological conditions encountered in captive wild

mammals. *Indian Journal of Animal Sciences*, 64(12), pp.1346–1347.

Slaffer, S.N. & Allchurch, A.F., 1995. Diagnosis and treatment of dilated (congestive) cardio-myopathy in a Sumatran orang-utan *Pongo pygmaeus abelii*. *Dodo*, p.147–152.

Sleeper, M.M. et al., 2014. Echocardiographic parameters of clinically normal adult captive chimpanzees (*Pan troglodytes*). *Journal of the American Veterinary Medical Association*, 244.

Sleeper, M.M., 2009. Is it acute heart failure or sudden cardiac death in the chimpanzee? *Journal of Medical Primatology*, 38(2), p.75; author reply 76.

Sleeper, M.M. et al., 2005. Successful treatment of idiopathic dilated cardiomyopathy in an adult chimpanzee (*Pan troglodytes*). *Comparative Medicine*, 55, pp.80–84.

Sos, E. et al., 2007. Veterinary aspects of a Sumatran orang-utan (*Pongo pygmaeus abelii*) introduction in a zoo situation. *Erkrankungen der Zootiere*, 43, pp.71–73.

Species360, formerly known as ISIS, 2005. Single Population Analysis and Records Keeping System (SPARKS): Version 1.5.

Steinetz, B.G. et al., 1996. Lipoprotein profiles and glucose tolerance in lean and obese chimpanzees. *Journal of Medical Primatology*, 25, pp.17–25.

Steinmetz, H.W. et al., 2007. Functional endoscopic sinus surgery in a Sumatran orang-utan (*Pongo pygmaeus abeli*). In H. Wibbelt, G.; Bergholz, N.; Seet, S.; Hofer, ed. *Proceedings of the Institute for Zoo and Wildlife Research, Berlin (7)*. Institute for Zoo and Wildlife Research, pp. 74–75.

Stevens, J.M.G. et al., 2007. Sex differences in the steepness of dominance hierarchies in captive bonobo groups. *International Journal of Primatology*, 28(6), pp.1417–1430.

Stringer, E.M. et al., 2010. Medical and surgical management of reproductive neoplasia in two western lowland gorillas (*Gorilla gorilla gorilla*). *Journal of Medical Primatology*, 39, pp.328–335.

Strong, V. et al., 2016. A systematic review of the literature relating to captive great ape morbidity and mortality. *Journal of Zoo and Wildlife Medicine*, 47(3), pp.697–710.

Struck, K. et al., 2007. Attempting to reduce regurgitation and reingestion in a captive chimpanzee through increased feeding opportunities: a case study. *Lab Animal*, 36(1), pp.35–38.

Sun, Y. & Weber, K.T., 2005. Animal models of cardiac fibrosis. *Methods in molecular medicine*, 117, pp.273–290.

Surbeck, M. & Hohmann, G., 2013. Intersexual dominance relationships and the influence of leverage on the outcome of conflicts in wild bonobos (*Pan paniscus*). *Behavioral Ecology and Sociobiology*, 67(11), pp.1767–1780.

Szentiks, C.A. et al., 2009. Lethal pneumonia in a captive juvenile chimpanzee (*Pan troglodytes*) due to human-transmitted human respiratory syncytial virus (HRSV) and infection with *Streptococcus pneumoniae*. *Journal of Medical Primatology*, 38(4), pp.236–240.

Thavendiranathan, P. et al., 2013. Improved interobserver variability and accuracy of echocardiographic visual left ventricular ejection fraction assessment through a self-directed learning program using cardiac magnetic resonance images. *Journal of the American Society of Echocardiography*, 26(11), pp.1267–1273.

Thiene, G. et al., 2000. Pathology and pathogenesis of arrhythmogenic right ventricular cardiomyopathy. *Herz*, 25(3), pp.210–5.

Tidholm, A. et al., 2001. Canine idiopathic dilated cardiomyopathy. Part I: aetiology, clinical characteristics, epidemiology and pathology. *The Veterinary Journal*, 162(2), pp.92–107.

Tobias, J.D. & Leder, M., 2011. Procedural sedation: a review of sedative agents, monitoring, and management of complications. *Saudi Journal of Anaesthesia*, 5(4), pp.395–410.

Tong, L.J. et al., 2013. Fatal arrhythmogenic right ventricular cardiomyopathy in 2 related subadult chimpanzees (*Pan troglodytes*). *Veterinary Pathology*, 51(4), pp.858–867.

Trupkiewicz, J. et al., 1995. Cerebral infarction associated with coarctation of the aorta in a lowland gorilla (*Gorilla gorilla gorilla*). *Journal of Zoo and Wildlife Medicine*, 26(1), pp.123–131.

Tsang, S., et al., 2007. Testosterone and cardioprotection against myocardial ischemia. *Cardiovasc.Hematol.Disord.Drug Targets.*, 7(1871–529X (Print) LA–eng PT–Journal Article PT–Research Support, Non–U.S. Gov't PT–Review), pp.119–125.

Unwin, S., et al., 2013. Management of severe respiratory tract disease caused by human respiratory syncytial virus and *Streptococcus pneumoniae* in captive chimpanzees (*Pan troglodytes*). *Journal of Zoo and Wildlife Medicine*, 44(1), pp.105–115.

Varki, N. et al., 2009. Heart disease is common in humans and chimpanzees, but is caused by different pathological processes. *Evolutionary Applications*, 2(1), pp.101–112.

Videan, E.N. et al., 2011. Diagnosis and prevalence of uterine leiomyomata in female chimpanzees (*Pan troglodytes*). *American Journal of Primatology*, 73, pp.665–670.

Videan, E.N., et al., 2007. Development of guidelines for assessing obesity in captive chimpanzees (*Pan troglodytes*). *Zoo Biology*, 26, pp.93–104.

Vielgrader, H. et al., 2005. Management of an outbreak of shigellosis in the Vienna Zoo. In K. Frolich & F. Steinbach, eds. *Proceedings of the Institute for Zoo and Wildlife Research, Berlin. No 766*. Prague, pp. 164–166.

Vignola, P.A. et al., 1977. Interobserver variability in echocardiography. *Journal of Clinical Ultrasound : JCU*, 5(4), pp.238–42.

Virmani, R. et al., 2001. Sudden cardiac death. *Cardiovascular Pathology*, 10(5), pp.211–218.

de Vries, A. et al., 2016. Clinical effects and pharmacokinetic variables of romifidine and the peripheral α2-adrenoceptor antagonist MK-467 in horses. *Veterinary Anaesthesia and Analgesia*, 43(6), pp.599–610.

de Wachter, P. et al., 2003. Ebola and the decline of gorilla (*Gorilla gorilla*) and chimpanzee (*Pan troglodytes)* populations in Minkebe Forest, north-eastern Gabon. *Oryx*, 37.

Walsh, P.D. et al., 2003. Catastrophic ape decline in western equatorial Africa. *Nature*, 422, pp.611–614.

WAZA, 2016a. Conservation Breeding Programmes: WAZA: World Association of Zoos and Aquariums. Available at: http://www.waza.org/en/site/conservation/conservation-breeding-programmes [Accessed August 16, 2016].

WAZA, 2016b. Conservation through Zoos and Aquariums: WAZA: World Association of Zoos and Aquariums. Available at: http://www.waza.org/en/site/conservation [Accessed August 16, 2016].

Wharton, G. et al., 2012. A minimum dataset for a standard transthoracic echocardiogram. echo research and practice, 2 (1), G9-G24

Whittaker, P. et al., 1989. Analysis of healing after myocardial infarction using polarized light microscopy. *The American Journal of Pathology*, 134(4), pp.879–93.

Wich, S. a et al., 2009. Captive and wild orangutan (Pongo sp.) survivorship: a comparison and the influence of management. *American Journal of Primatology*, 71(8), pp.680–6.

Williams, J.M. et al., 2008. Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. *American journal of primatology*, 70(8), pp.766–77.

Wilms, T. & Bender, U., 2015. *International studbook for the western lowland gorilla (Gorilla g.gorilla) (Wyman, 1847-) 2014*, Frankfurt Zoo 2015.

Wolfla, C.E. et al., 2004. Reversal of neurologic deficit in an adult gorilla with severe symptomatic lumbar stenosis. *Spine*, 29, pp.E95–E97.

World Health Federation, 2017. Cardiovascular disease risk factors: World Heart Federation. Available at: http://www.world-heart-federation.org/cardiovascular-health/cardiovascular-disease-risk-factors/ [Accessed February 21, 2017].

World Health Organisation, 2011. About cardiovascular diseases. World Health Organisation, Geneva. Available from: http://www.who.int/cardiovascular_diseases/about_cvd/en/

World Health Organisation, 2010. International statistical classification of diseases and rrelate health problems 10th Revision. World Health Organisation, Geneva. Available at: http://apps.who.int/classifications/icd10/browse/2010/en.

World Health Organisation, 2006. Neonatal and perinatal mortality: country, regional and global estimates. In WHO Press, World Health Organization Geneva. Available from: http://apps.who.int/iris/bitstream/10665/43444/1/9241563206_eng.pdf

World Health Organisation, 2014. Health Topics: Epidemiology. Available from: http://www.who.int/topics/epidemiology/en/ [Accessed February 20, 2017]

Yamaji, K. et al., 2005. Apoptotic myocardial cell death in the setting of arrhytmogenic right ventricular cardiomyopathy. *Acta Cardiologica*, 60(5), pp.465–470.

Yeo, D.S.-Y. et al., 2013. A highly divergent encephalomyocarditis virus isolated from nonhuman primates in Singapore. *Virology Journal*, 10(1), p.248.

Zimmermann, N. et al., 2009. Computertomographic evaluation of the upper respiratory tract in orang-utans (*Pongo pygmaeus*, *Pongo abelii*). In G. Wibbelt et al., eds. *Proceedings of the International Conference on Diseases of Zoo and Wild Animals 2009, May 20th - 24th, 2009, Beekse Bergen/The Netherlands*. pp. 102–103.

Zimmermann, N. et al., 2011. Upper respiratory tract disease in captive orangutans (*Pongo* sp.): prevalence in 20 European zoos and predisposing factors. *Journal of Medical Primatology*, 40(6), pp.365–75.

Zoller, M. et al., 2005. Cerebral calcinosis in a young chimpanzee. In F. Frolich, K.; Steinbach, ed. *Proceedings of the Institute for Zoo and Wildlife Research, Berlin (6)*. Prague: Institute for Zoo and Wildlife Research, pp. 112–114.

APPENDICES

Appendix 1: Literature search terms for all databases

Database	Term	Free text terms	Subject headings and map terms
CAB Abstracts in OVID	***Great ape***	ape or apes or gorilla* or chimpanzee* or chimp or pan troglodyte* or bonobo* or pan paniscus or orangutan* or orang-utan* or orang utan* or pongo or pongidae	gorilla, pan, pongo
	Morbidity & mortality	mortality or mortalities or death* or fatal* or epidemiolog* or surviv* or necropsy or necropsies or autopsy or autopsies or post mortem* or post-mortem* or post-mortem* or morbidity or disease* or patholog*	mortality, "causes of death", death, epidemiology, fatal infections, survival, postmortem examinations, postmortem changes or pathology (animal pathology or histopathology or immunopathology or physiopathology or diseases or pathogenicity or pathogens or virulence factors not plant pathology) morbidity, diseases
	Captivity	captive or captivity or zoo*	zoo animals, zoological gardens
Zoological Record in Ovid (up to 2007)	***Great ape***	ape or apes or gorilla* or chimpanzee* or chimp or pan troglodyte* or bonobo* or pan paniscus or orangutan* or orang-utan* or orang utan* or pongo or pongidae	pongidae
	Morbidity & mortality	mortality or mortalities or death* or fatal* or epidemiolog* or necropsy or necropsies or autopsy or autopsies or post mortem* or post-mortem* or postmortem* or morbidity or morbidities or disease* or patholog*	mortality, diseases and disorders, epidemiology
	Captivity	captive or captivity or zoological or zoo	zoos and wildlife parks, zoological gardens, care in captivity, survival in captivity, captive breeding and rearing records, captive breeding records
Web of Knowledge[1]	***Great ape***	"great ape" or gorilla* chimpanzee* OR chimp OR pan troglodyte* bonobo* OR pan paniscus orangutan* OR orang-utan* OR orang utan* OR pongo or pongidae	
	Morbidity & mortality	mortality or mortalities or death* or fatal* or epidemiolog* or necropsy or necropsies or autopsy or autopsies or post mortem* or post-mortem* or post-mortem* or patholog* or disease* or morbidity or morbidities	
	Captivity	captivity or captive or zoological or zoo	

[1] *Web of knowledge coverage; Web of Science Core Collection, BIOSIS Citation Index, BIOSIS Previews, Current Contents Connect, Data Citation Index, Derwent Innovations Index, MEDLINE, SciELO Citation Index, Zoological Record 2007 onwards*

Appendix 2: Literature review article inclusion and exclusion criteria

Term	Inclusion	Exclusion
Great ape	Includes conditions relating specifically to at least one of the four great ape taxa; bonobo, chimpanzee, gorilla or orangutan	All other species (including lesser apes, i.e. gibbons) Study of pathogens from evolutionary or zoonotic (human) perspective
Captivity	Includes animals in zoological collections and sanctuaries with no contact with wild animals of the same species	Free-living or wild animals Animals in rehabilitation centres Semi-wild animals Pets
Morbidity/ mortality	The reporting of a naturally occurring disorder, disease or infection Reporting of trends/patterns of morbidity or mortality, or general demographic information	Experimental infection Reporting of 'normal' findings, e.g. blood parameters, heart rate, echocardiographic parameters
Clinical significance	The reporting of a clinical event (i.e. illness, surgical, intervention, behavioural abnormality or death) from a veterinary perspective	Mere pathogen presence (serology, faecal parasite screen). Discussion of the following without reporting of a specific clinical case: use of a diagnostic test, behavioural response to illness/death, training of an animal for diagnostic test/administering therapy

Appendix 3: Codes and definitions of categories used for classification of articles according to aetiology/pathological process

Aetiology/pathological process	Code	Definition
Degenerative	DG	Disorder characterised by progressive deterioration in tissue function or structure over time often due to normal bodily wear; includes dental disease and osteoarthritis
Congenital/ hereditary	CO	Disorder or condition which is present at birth, or an abnormality which has been genetically inherited; includes chromosomal abnormalities
Metabolic	ME	Disorder characterised by abnormal function of the body's metabolism and homeostatic control; includes disorders of the endocrine system and obesity
Neoplastic	NE	Disorder characterised by the uncontrolled growth of tissue; a tumour
Infection	IN	Disorder caused by invasion of the body by pathogenic microorganisms; includes bacterial, viral, parasitic and fungal infections
Idiopathic	ID	A disorder which arises spontaneously through an unknown of obscure cause
Immunological	IM	A disorder characterised by abnormal function of the body's immune responses; includes immune mediated conditions and allergies
Trauma/accident	TR	Disorder characterised by an unexpected event, or extrinsic agent, causing injury caused to part of the body; does not include self-trauma
Husbandry-related	HU	Disorders caused by management practices, or the animal's diet, social situation, housing or environment
Behavioural/ psychological	BE	Disorder characterised by an abnormal psychological state or behaviour which differs from the norm for that individual or species; includes self-trauma
Vascular	VA	Disorder primarily affecting the blood vessels; includes thromboembolism, cerebrovascular accident, hypertension, ischemic heart disease and aneurysms
Miscellaneous	MI	A disorder for which the underlying pathological process does not fit into any of the above categories
Unknown	UK	A disorder for which a cause or underlying pathological process could not be identified

Appendix 4: Codes and definitions of categories used for classification of articles according to body system

Body system	Code	Definition
Skin	SK	The integumentary system including the external ear canal and buccal (non-gingival) mucosa
Musculoskeletal	MS	The skeleton or skeletal muscles
Neurological	NE	The central or peripheral nerves; includes conditions of the brain and spinal cord
Ophthalmic	OP	The eyes, eyelids, tear ducts and associated structures
Urinary	UR	The lower urinary tract; includes the bladder and urethra
Renal	RE	The upper urinary tract; includes the kidneys and ureters
Reproductive	RP	Male or female reproductive tract; includes problems of pregnancy, parturition and lactation
Gastrointestinal	GI	The gastrointestinal tract; includes the exocrine pancreas
Hepatobiliary	HB	The liver, gall bladder and associated structures
Cardiovascular	CV	The cardiovascular system; includes the heart, pericardium and blood vessels
Respiratory	RS	The respiratory system; includes lungs, trachea, nasal passages and sinuses
Haematopoietic/ lymphatic	HL	The haematopoietic or lymphatic system; includes disorders of blood cells, bone marrow and lymph nodes
Endocrine	EN	The endocrinological system; includes disorders of the pituitary, thyroid or adrenal glands or endocrine pancreas which result in hormonal imbalance
Dental	DE	The teeth, gingiva and related structures
Behavioural	BE	Any activity judged to be outside the normal behaviour pattern for individuals of that signalment, where no underlying medical or physiological causal abnormality can be found
Generalised/ multi-system/other	GM	Includes disorders which affect more than one body system, or those which cause generalised (i.e. systemic) disease. Includes those disorders which cannot be categorised as any of the above (e.g. those affecting the mesentery)

Appendix 5: Classification system used for categorisation by cause of death: includes relevant codes, definitions and equivalent ICD-10 categories (WHO 2010)

Title	Code	Equivalent ICD-10 Category	Definition
Diseases of the ear (auditory)	AU	VIII	Diseases of the ear and mastoid process. *Includes external, middle, and inner ear and mastoid process*
Mental and behavioural disorders	BE	V	Mental and behavioural disorders. *Includes mood (affective) disorders, neurotic, stress-related and somatoform disorders, behavioural syndrome associated with physiological disturbances and physical factors, disorders of psychological development, and of personality and behaviour.*
Diseases of the circulatory system	CI	IX	Diseases of the circulatory system. *Includes heart disease (all forms), hypertensive diseases, ischemic heart diseases, diseases of the pulmonary circulation, cerebrovascular diseases, diseases of the arteries, arterioles, capillaries, veins, lymphatic vessels and lymph nodes (if not classified elsewhere)*
Congenital malformations, deformations & chromosomal abnormalities	CO	XVII	Congenital malformations, deformations and chromosomal abnormalities
Endocrine, nutritional and metabolic	EN	IV	Disorders of the endocrine or metabolic systems, and nutritional deficiencies. *Include disorders of the thymus, thyroid, adrenal, parathyroid and pituitary glands, diabetes mellitus and other disorders of glucose regulation, nutritional deficiencies, malnutrition, obesity, metabolic disorders and dysfunction of the ovaries/testicles*
External causes	EX	XX	External causes of mortality. *Includes trauma, injury, foreign body penetration, burns, poisoning and complications of surgical or medical care not classified elsewhere*
Diseases of the digestive system	DI	XI	Diseases of the digestive system. *Includes oral cavity, salivary glands, jaws, oesophagus, stomach, duodenum, appendix, non-infective enteritis/colitis, other diseases of the intestines, peritoneum, liver, gall bladder, biliary tract and pancreas*
Diseases of the genitourinary system	GU	XIV	Diseases of the genitourinary system. *Includes glomerular disease, renal tubulo-interstitial diseases, renal failure, urolithiasis, other disorders of the kidney and ureter, disorders of the male and female genital organs, the breast, female pelvic organs*

Diseases of the blood and blood forming organs and certain disorders involving the immune mechanism	HI	III	Diseases of the blood and blood forming organs and certain disorders involving the immune mechanism. *Includes anaemias, coagulation defects, disorders of the spleen and certain immunodeficiency disorders. Excludes autoimmune and infectious (e.g. viral) immunodeficiency disorders.*
Infectious & parasitic disease	IN	I	Disease generally recognised as communicable or transmissible. Includes conditions of bacterial, viral, fungal or parasitic cause. *Excludes carriers of disease, certain localised infections (see body system related categories), infections specific to the perinatal period and acute respiratory infections*
Diseases of the skin & subcutaneous tissue	SK	XII	Diseases of the skin and subcutaneous tissue. *Includes infections of the skin and subcutaneous tissue, dermatitis, urticaria*
Diseases of the musculoskeletal system & connective tissue	MS	XIII	Diseases of the musculoskeletal system and connective tissue. *Includes arthropathies, soft tissue disorders, osteopathies and chondropathies*
Neoplastic	NE	II	Malignant or benign neoplasms affecting any body system. *Includes functional neoplasms which have additional metabolic or endocrine effects.*
Disease of the nervous system	NS	VI	Diseases of the nervous system. *Includes central and peripheral nervous system*
Diseases of the eye & adnexa (ophthalmic)	OP	VII	Diseases of the eye and adnexa. *Includes diseases of the eyelid, lacrimal system, orbit, conjunctiva, globe, optic nerve and visual pathways, ocular muscles and visual disturbances*
Certain conditions originating in the perinatal period	PE	XVI	Certain conditions originating in the perinatal period (even if death results later). *For example, conditions related to gestation length or foetal growth, arising due to complications of pregnancy, labour or delivery and birth trauma. Includes stillbirths and abortions.*
Pregnancy, parturition & the puerperium	PP	XV	Conditions related to or aggravated by pregnancy, childbirth or puerperium (maternal causes or obstetric). *Includes abortion and complications of delivery and labour.*
Diseases of the respiratory system	RE	X	Disorders of the respiratory system. *Includes acute respiratory infections, influenza, pneumonia, disorders of the upper and lower respiratory tract, disorders of the pleura or interstitium*
Other/ miscellaneous	OM	XVIII	Symptoms, signs and abnormal clinical and laboratory findings not classified elsewhere

Appendix 6: Number of animals included and excluded from age at death and cause of death analysis

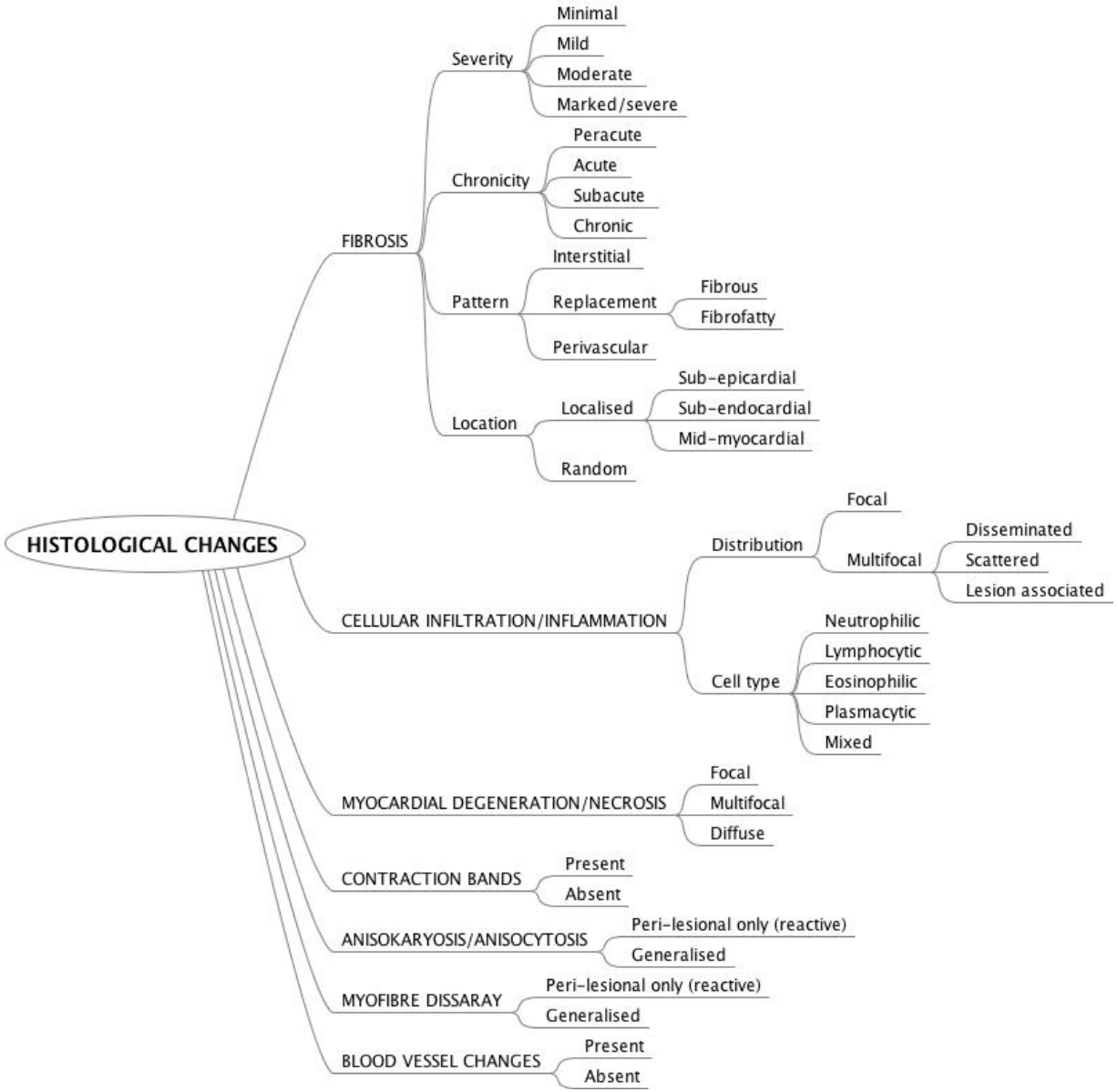
			BONOBO	CHIMPANZEE	GORILLA	ORANGUTAN
NUMBER OF DEATHS ***(total, n)***			***47***	***370***	***151***	***113***
AGE AT DEATH ANALYSIS	***Excluded***	Insufficient data, *n*[1]	0	14	8	6
		Foetal deaths, *n*	8	35	14	10
	Included		***39***	***321***	***129***	***97***
CAUSE OF DEATH ANALYSIS	***Excluded***	Data not provided, *n*	1	98	23	34
		Cause of death unknown, *n*	0	12	4	3
	Included		***38***	***211***	***102***	***60***

[1] *Insufficient data provided to allow for categorisation by age at death, including for example those animals that died aged 0 days but for which post-mortem information enabling foetal and perinatal deaths to be distinguished between was not available*

Appendix 7: Table showing proportional mortality associated with each cause of death, for all four taxa, divided by age category (PE: perinatal; IN: infant; JU: juvenile: SA: sub-adult/adolescent; AD: adult; AG: aged/elderly; T: total). *Most frequently identified cause(s) of death for each taxa/age category are shown in blue. Behav./mgt.: behavioural/management; Musculosk.: muculoskeletal; Preg/ part.: associated with pregnancy/parturition*

	BONOBO							**CHIMPANZEE**							**GORILLA**							**ORANGUTAN**						
	PE	***IN***	***JU***	***SA***	***AD***	***AG***	***T***	***PE***	***IN***	***JU***	***SA***	***AD***	***AG***	***T***	***PE***	***IN***	***JU***	***SA***	***AD***	***AG***	***T***	***PE***	***IN***	***JU***	***SA***	***AD***	***AG***	***T***
Behav./mgt.										3	3	2		***8***							***0***							***0***
Circulatory					9	4	***13***			1	6	15	15	***37***					7	8	***15***					2	4	***6***
Congenital														***0***							***0***			2				***2***
Digestive										2	1	9	2	***14***		4	1	1	10	7	***23***			1	1	3		***5***
Endocrine													1	***1***					1		***1***							***0***
External	2		3		1		***6***	15	11	11	8	8	8	***61***	4	8	3				***15***	1	3	1	1	5	1	***12***
Genitourinary						1	***1***					5	5	***10***				1	3	2	***6***							***0***
Infectious					2		***2***			1	3	3		***7***		1		4	2	2	***9***		1		3		1	***5***
Misc./ other													6	***6***						1	***1***						2	***2***
Musculosk.												2	2	***4***						1	***1***							***0***
Neoplastic				1			***1***					2	3	***5***			1		1	6	***8***					2	5	***7***
Neurological		1					***1***		1	1		3		***5***					1	1	***2***						1	***1***
Perinatal	2						***2***	13	5					***18***	10						***10***	1						***1***
Preg/ part.											2			***2***				1			***1***							***0***
Reproductive											1			***1***							***0***					3		***3***
Respiratory	1	4	1	3	2	1	***12***		11	12	4	1	4	***32***		4	2	1	1	2	***10***		2	2	3	6	3	***16***
TOTAL	**5**	**5**	**4**	**4**	**14**	**6**	***38***	**28**	**28**	**31**	**28**	**50**	**46**	***211***	**14**	**17**	**7**	**8**	**26**	**30**	***102***	**2**	**6**	**6**	**8**	**21**	**17**	***60***

Appendix 8: Flow diagram summarising results of thematic analysis, demonstrating the wide variety of histological changes which were reported to be features of 'fibrosing cardiomyopathy' (FCM) in the post-mortem reports reviewed



Appendix 9: Protocol for ante-mortem (echocardiographic) assessment of the great ape heart

Great Ape TAG - endorsed

A Guide to Performing a Complete Standardised Echocardiographic Examination

In Great Apes

April 2015

Victoria Strong BVSc BSc (Hons) MRCVS
Mike Martin MVB DVC MRCVS, RCVS Specialist in Veterinary Cardiology
Sarah Chapman BVM&S MSc CertZooMed MRCVS
Malcolm Cobb MA Vet MB DVC PhD MBA FHEA MRCVS
Sharon Redrobe BVetMed BSc (Hons) DZooMed MRCVS
With the support of the Great Ape Heart Project (Zoo Atlanta, USA)

Ape Heart Project (EAZA Great Ape TAG Endorsed)
Twycross Zoo, Burton Road, Atherstone, Warwickshire, UK, CV9 3PX
Website: www.twycrosszoo.org/ape-heart-project.aspx *Email:* heartproject@twycrosszoo.org

GUIDELINES

A Guide to Performing a Complete Standardised Echocardiographic Examination in Great Apes

V. Strong[1,2], M. Martin[3], S. Chapman[2], M. Cobb[1], S. Redrobe[2]

[1]School of Veterinary Medicine & Science, University of Nottingham, Sutton Bonington, LE12 5RD
[2]Twycross Zoo, Burton Road, Atherstone, Warwickshire, CV9 3PX
[3] Veterinary Cardiorespiratory Centre, 43 Waverley Road, Kenilworth, Warwickshire, CV8 1JL

INTRODUCTION

This guide has been created as part of the EAZA Great Ape TAG endorsed Ape Heart Project. Based at Twycross Zoo (UK), the project is a Europe wide collaborative initiative striving to achieve a better understanding of great ape cardiovascular disease.

It is an ambition that all veterinarians, cardiologists and sonographers will find these guidelines useful when performing echocardiographic examinations on great apes.

The purpose of this protocol is to;

- Promote consistency and quality in performing cardiac assessment of great apes between different institutions and by multiple individuals
- Ensure that the same techniques are used when collecting data, thereby allowing comparison between information gathered from various sources
- Facilitate individual clinical assessment and disease screening

The echocardiographic examinations performed will aid in ***clinical assessment*** and therefore benefit the individual animals concerned. We ask that each examination is also submitted to the Great Ape Heart Project ***database***. Information being gathered from this database will aid and improve the future diagnosis and management of great ape cardiovascular disease worldwide.

SECTION 1: General Information

a. Identifying Information

All data collected should be clearly labelled with patient identifying information, including the following:

- Studbook number
- Species (and subspecies if known)
- Individual institution ID (name/number)
- Zoological collection (at the time of examination)
- Date of birth

Further information as requested in the cardiovascular examination form should also be provided. It is likely that the supervising zoo veterinarian will need to complete these fields:

- Examination date
- Sonographer/cardiologist name and contact details
- Weight
- Body measurements (see form for details)
- Body weight
- Current health problems and medications

b. Anaesthesia

These guidelines are written with the assumption that cardiac examination is being performed under anaesthesia. All anaesthetic drugs can affect the cardiovascular system. Detailed information regarding any drugs given, including doses and timings of administration should accompany every cardiac examination submitted to the database.

Some zoos are performing awake echocardiography and blood pressure assessment in great apes – if you are interested in receiving more information please contact us.

c. Examination submission

Following assessment, details of the examination (as requested in the cardiac examination form) should be submitted to the Great Ape Heart Project database. For details on how to do this, please see their website.[3] Once a *complete* exam has been submitted a cardiac advisor will review the scan and provide comments and image quality feedback within 4-6 weeks*. This information will then be shared with the European team.

**If the animal is sick at the time of examination and you require a more rapid response, please communicate this directly with the GAHP at the time of exam submission*

d. Data storage and submission

Echocardiographic studies should be recorded as moving images (at least 3 cardiac cycles) and stored in a medium which allows for video playback. Stationary images, e.g. spectral Doppler studies and ECG strips can be provided as .jpeg images. The submitting institution should also retain original files for their reference.

SECTION 2: A Protocol for Performing a Standard Transthoracic Echocardiogram

This protocol aims to provide a guideline for performing a transthoracic echocardiogram (TTE) in great apes. The concept is based upon the British Society of Echocardiography Minimum Dataset for humans[2].

This protocol provides a recommended sequence for performing a systematic study.

Please note that following this protocol provides a basic dataset only. This can be supplemented with additional images as indicated in the presence of abnormal pathology.

This protocol is not a final or definitive guide, but a consensus view that should evolve and adapt with the opinions of other experts in the field and as our knowledge develops and progresses.

a. Technical information

A standard **2-5MHZ transducer** is adequate in most cases for adults. For smaller individuals, a 5-10MHz transducer may be more appropriate.

Whilst not all sonographers have access to the technology for performing tissue Doppler, inclusion of this data in echocardiographic assessment is ideal and the collection of appropriate images and data are desirable.

b. Animal Preparation & Positioning

Echocardiographic examination is aided by placement of the animal in left lateral recumbency with the left arm above the shoulder (see Figure 1, right):

It is possible to perform the scan with the animal in dorsal recumbency if necessary.

The use of padding underneath the animal is recommended to prevent injury.

Images can be obtained by parting of the hair and application of gel; clipping is rarely required.

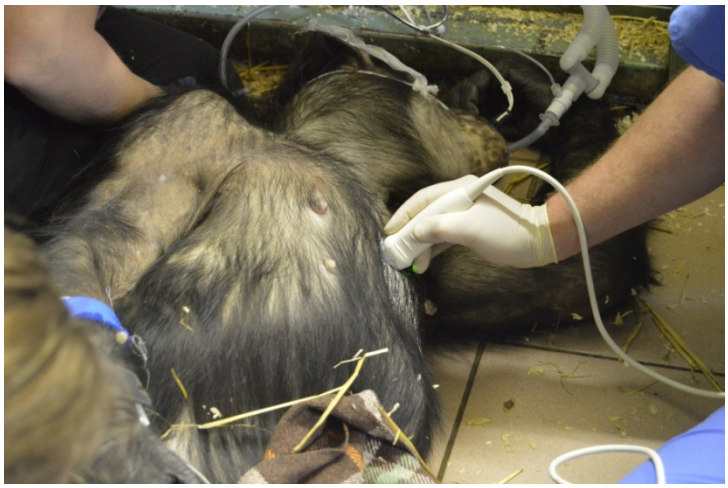


Figure 8: Chimpanzee positioned in left lateral recumbency for echocardiographic examination

c. Acoustic Windows

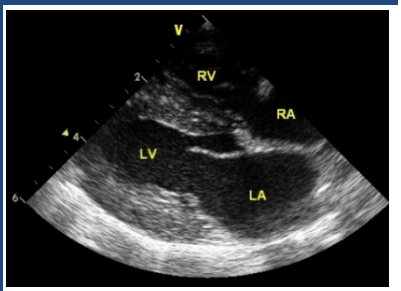
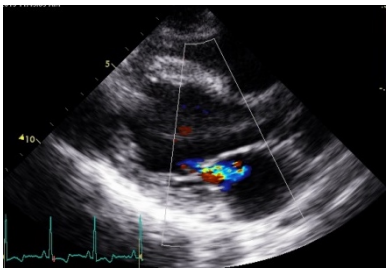
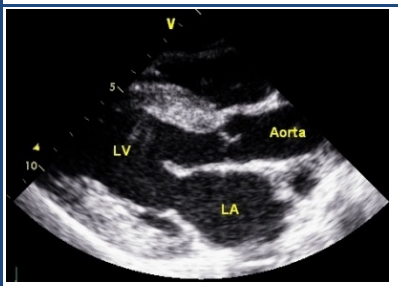
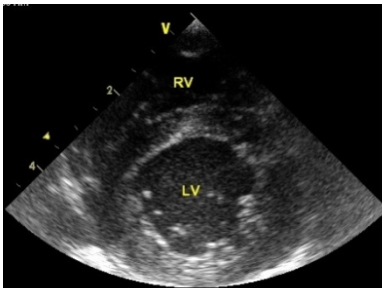
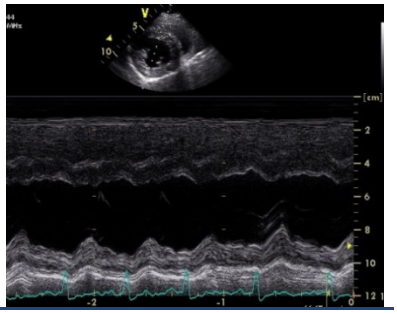
Details regarding acoustic window location are provided in Table 1 below:

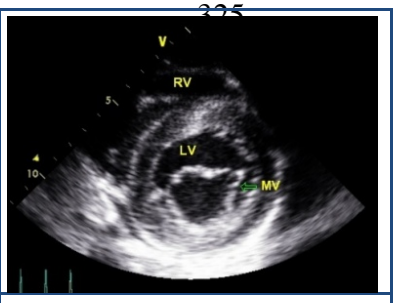
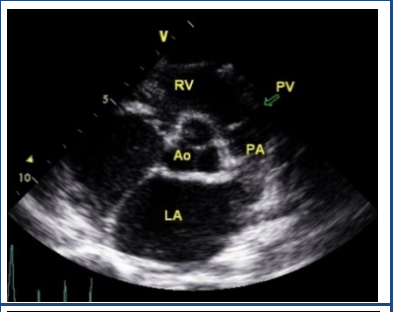
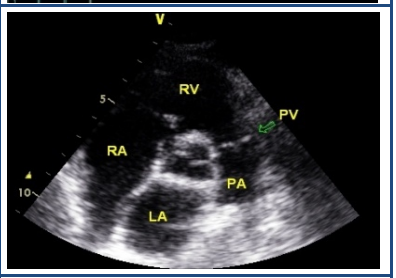
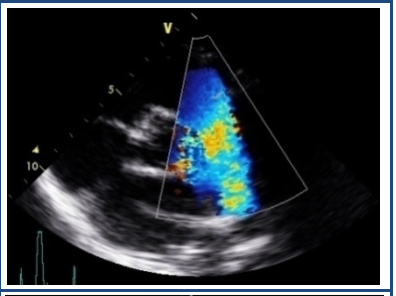
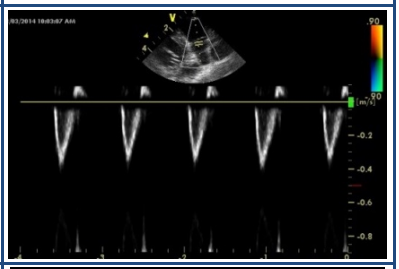
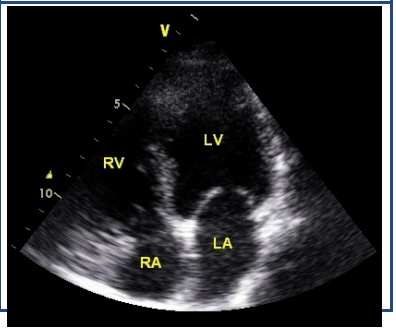
VIEW	ABBREVIATION	TRANDSUCER LOCATION	THUMB/NOTCH POSITION
Parasternal long axis	PLAX	3rd-5th ICS, close to sternum	Towards right shoulder, 10-11 o'clock position
Parasternal short axis	PSAX	Rotate transducer 90˚ from PLAX position	Towards left shoulder, 2-3 o'clock position
Apical four chamber	A4C	Left lateral chest, 5-6th ICS, just below nipple, beam angled towards head	Towards left shoulder, 2-3 o'clock position
Apical five chamber	A5C	Tilt cranially from A4C view	As above
Apical two chamber	A2C	Rotation clockwise from A4C	As above

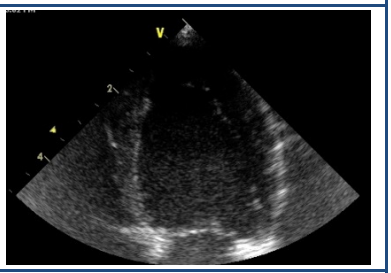
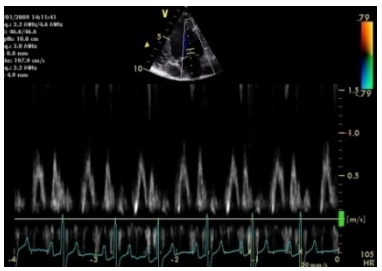
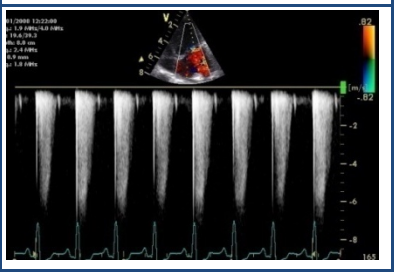
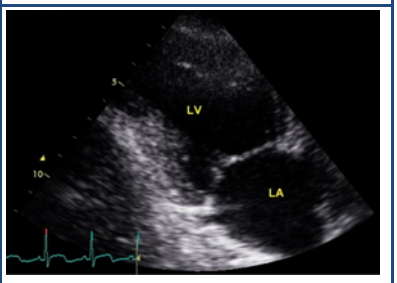
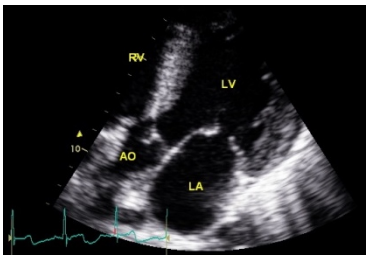
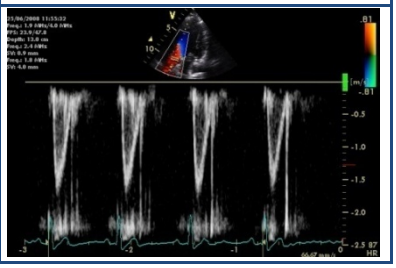
Table 1:
Table showing a guide to acoustic window positioning for obtaining standardised echocardiographic images
(ICS = intercostal space)

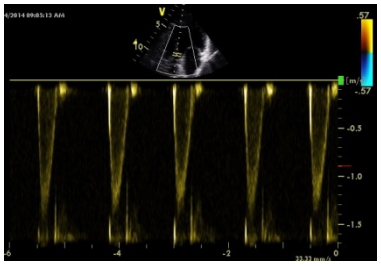
d. Image orientation

Please orientate the images as per the examples shown overleaf.

VIEW	MODALITY	VIEWS TO OPTIMISE	MEASUREMENTS	EXPLANATORY NOTE	SAMPLE IMAGE
PLAX	2D	LV, LA, MV		Ensure whole of LV and LA are visible Assess LV chamber size & check for any region wall motion abnormalities	
			LA diameter (end ventricular systole, inner dimension)	Assess LA appearance and size. Assess MV appearance, structure and function	
		RA, RV (Adjust depth to optimise for right heart)		Assess RV & RA chamber sizes	
	2D + CFM			Screen for MV regurgitation Screen for TV regurgitation	
	2D	Ao + LV		Assess appearance of AV/LVOT	
	2D +CFM			Screen for outflow turbulence and aortic regurgitation	
PSAX	2D	Screen LV from base to apex	LVIDd/s, IVSd, LVPWd in systole and diastole (2D or M-mode)	Ensure symmetry of papillary muscles and chordae tendineae Ensure endocardium of RV septum is visible	
			Measurements can alternatively be performed in PLAX view, if this images better	Assess LV chamber size, function and wall thickness & check for any region wall motion abnormalities, scanning from apex to base	
	M mode			M-mode throughLV at level of chordate	

PSAX contd.	2D 2D + CFM	MV		Assess MV structure and function Screen for MV regurgitation	
	2D	Aortic valve	Ao diameter	Ensure symmetry and good visualisation of 3 aortic valve cusps Assess structure of AV; measure AV diameter	
		LA	Max LA diameter & AV:LA ratio	Assess appearance of LA	
		PV		Ensure maximal length of RVOT to pulmonary artery visualised Assess structure and function of RVOT, PV& PA Assess appearance of RA & RV	
	2D + CFM	PV		Screen for PV turbulence and regurgitation	
	CW	PV regurgitation	PR V_{max}	If present; measure peak flow velocity of regurgitant jet	
	PW	PV velocity	V_{max}	Measure peak flow velocity	
A4C	2D	All 4 chambers MV, TV	MV EDV/ESV	Ensure endocardium is visible throughout LV(including apex) Assess LV chamber size, LV wall thickness &check for any region wall motion abnormalities Assess structure and function of MV and TV	

	2D + CFM			Screen for MV and TV regurgitation	
A4C contd.	2D	LV (adjust depth to increase image size)	Measure LV volume (Simpson's rule) in diastole and systole and EF%)	Ensure endocardium is visible throughout (including apex). Measure LV volumes (systole and diastole)	
	PW	Mitral inflow Tricuspid inflow	Peak E &A wave velocities	Place cursor at level of MV leaflet tips and record inflow velocity Place cursor at level of TV leaflet tips and record inflow velocity	
	CW	MV or TV regurgitation	Peak velocity of any MR	If present; measure flow velocity of the regurgitant jet	
A2C	2D	LV	(Optional: LV volume in diastole & systole)	Ensure endocardium visible throughout (including apex). Assess LV chamber size and wall thickness & check for any regional wall motion abnormalities	
A5C	2D 2D +CFM	Ao& LV		Assess LV chamber size and wall thickness & check for any regional wall abnormalities Assess AV appearance & function Screen for AV turbulence & regurgitation	
	PW	AoV velocity	Peak Ao flow velocity	Place cursor just beyond AoVand record outflow velocity	

	CW	AoV regurgitation	Peak velocity AR	If present, measure flow velocity of regurgitant jet	

Overleaf is a shortened image checklist which may be a useful *aide memoir* to print off and refer to during an echocardiographic examination.

Checklist of information to be gathered during anaesthetic echocardiographic examination

MEASUREMENTS			
Weight (kg):		**Body condition score (1-9):**	
Crown-rump length (cm):			

BLOOD PRESSURE (During echocardiogram)		✓
Start	Systolic, diastolic and mean	
End	Systolic, diastolic and mean	

ECHOCARDIOGRAPHY			
View	**Modality**	**Views to optimise**	✓
PLAX	2D	LV LA MV	
	2D + CFM	MV	
	2D	Ao + LVOT	
	2D + CFM	AV	
	2D	RV RA	
	2D + CFM	TV	
PSAX	2D	LV (base to apex)	
	(M mode)	LV (level of chordae tendineae)	
	2D	MV	
	2D + CFM	MV	
	2D	AV LA RVOT + PV	
	2D + CFM	PV	
	CW*	PV regurgitation	
	PW	PV velocities	
A4C	2D	All 4 chambers	
	2D	MV	
	2D + CFM	MV	
	PW	Mitral inflow	
	CW*	MV regurgitation	
	2D	TV	
	2D + CFM	TV	
	PW	Tricuspid inflow	
	CW*	TV regurgitation	
	2D	LV (zoomed in for volume measurements)	
A2C	2D	LV + LA	
A5C	2D	Ao + LV	
	2D + CFM	AV	
	CW*	AV regurgitation	

	PW	Aortic velocities	

Acknowledgements:

With thanks to the Great Ape Heart Project (Zoo Atlanta, USA) for their assistance and support.

References:

[1] Cardiology Teaching Package: A Beginners Guide to Normal Heart Function, Sinus Rhythm & Common Cardiac Arrhythmias. University of Nottingham, School of Health Sciences; http://www.nottingham.ac.uk/nursing/practice/resources/cardiology/function/chest_leads.php

[2] Wharton G. et al. 2012. A Minimum Dataset for a Standard Transthoracic Echocardiogram: From the British Society of Echocardiography Education Committee (Wharton G. (lead author), Steeds R. (chair), Allen J., Brewerton H., Jones R., Kanagala P., Lloyd G., Masani N., Matthew T., Oxborough D., Rana B., Sandoval J., Smith N., and Wheeler R.)

[3] Great Ape Heart Project website: http://greatapeheartproject.org/

When performing a cardiac assessment on a great ape, please ensure that the **examination is submitted to the Great Ape Heart Project database.**

Further details and copies all of the forms and protocols can be downloaded from our project website (see below).

Very many thanks for supporting this project.

EAZA Great Ape TAG Endorsed Ape Heart Project

Website: www.twycrosszoo.org/ape-heart-project.aspx

Email: heartproject@twycrosszoo.org

Appendix 10: Part 1 of the post-mortem protocol (basic examination and sampling)

Great Ape TAG endorsed

A Protocol for Basic Post-Mortem Examination and Sampling of the Cardiovascular System

Of Great Apes

February 2015

Victoria Strong BVSc BSc (Hons) MRCVS
*Sharon Redrobe*BVetMed BSc (Hons) DZooMed MRCVS
Sarah Chapman BVM&S MSc CertZooMed MRCVS
Phillipa Dobbs BVSc MRCVS
Kerstin Baiker DVM DiplECVP

EAZA Great Ape TAG Endorsed Ape Heart Project
Website: www.twycrosszoo.org/ape-heart-project.aspx
Email: heartproject@twycrosszoo.org

GUIDELINES

A Protocol for Basic Post-Mortem Examination and Sampling of the Cardiovascular System of Great Apes

V. Strong[1,2], S. Redrobe[2], S. Chapman[2], P. Dobbs[2], K. Baiker[1]

[1]*School of Veterinary Medicine & Science, University of Nottingham, Sutton Bonington, LE12 5RD*

[2]*Twycross Zoo, Burton Road, Atherstone, Warwickshire, CV9 3PX*

1. Introduction

This protocol has been created as part of the EAZA Great Ape TAG endorsed Ape Heart Project. Based at Twycross Zoo (UK), the project is a Europe wide collaborative initiative striving to achieve a better understanding of great ape cardiovascular disease through a combination of epidemiological study, clinical and pathological investigation.

This protocol aims to provide a guideline for performing a systematic and comprehensive approach to the post-mortem examination and sampling of the cardiovascular system in great apes.

Cardiovascular system examination should form part of a whole body gross examination and histopathology; this protocol is therefore intended to be supplementary to the general Great Ape TAG Veterinary Guidelines for performing post-mortem examination.

The purpose of this protocol is to;

- Promote consistency and quality in post-mortem examination of the cardiovascular system in great apes
- Standardise and maximise information gathering
- Facilitate comparative study between post-mortem findings and relevant samples
- Permit consistent sampling of the heart for subsequent examination by a designated pathologist

It is our ambition that all veterinarians and pathologists will follow these guidelines when performing post-mortem examination of great apes within EAZA collections.

If you still wish to use your own pathologist for examination of the heart, please refer them to us for a copy of our full cardiac post-mortem examination protocol.

When post-mortem examination of the cardiovascular system is performed, it is requested that photographs are taken at all stages of the process, and in particular of any abnormalities.

If the abnormal accumulation of fluid is noted at any stage, it should be quantified (in ml, or weighed if clotted), characterised (colour, consistency, specific gravity) and where possible a sample stored.

2. Identifying Information

All information requested in the sample submission form should be provided. All photos, paperwork and samples must be clearly labelled with patient identifying information, including the following:

- Studbook number
- Species (and subspecies if known)
- Individual institution ID (name/number)
- Zoological collection
- Date of birth
- Date of death

3. Supporting Documents/Files

A copy of the full post-mortem report or summary of the findings elsewhere in the body should also be sent, where possible. Other documents of use are;

- Copy of the animal's clinical history
- A copy of the animal's records (e.g. ARKS/ZIMS report)
- Photographs taken during the post-mortem examination;
    - Ideally photographs should be taken at all stages of the examination
    - Photographs of any abnormalities found are of particular use

4. Sending Samples

Once the heart is in formalin, please contact the project co-ordinator at the email address below. Once the heart is fixed, remove it from the formalin (this can be re-used) and wrap it in saline soaked gauze swabs or similar to prevent desiccation. Double bag the sample and package suitably for postage/courier to the address below. Send all samples with a completed sample submission form.

If sending the sample from a zoological collection ***within the EU*** no CITES permit is required, but the sample should be accompanied by a letter detailing the nature of the contents (e.g. chimp post-mortem sample), and the reason for the transfer (i.e. research). If sending the sample from a zoo ***outside the EU*** additional advice from CITES should be sought.

5. Report of Findings

A preliminary report of macroscopic findings will be sent to the submitting zoo/vet within a week of receipt of the sample (by email or phone), and a full written report within 6 working weeks.

Protocol for Post-Mortem Examination and Sampling of the Cardiovascular System of Great Apes

STEP	ACTION	EXPLANATORY NOTE
1	Weigh the animal Body condition score Measure crown-rump length	Record the weight (in kg) BCS scale 1-5 From top of the head (crown) to the bottom of the buttocks
2	Open the chest and examine the thorax	Note appearance of lungs, pleural cavity etc. Assess for presence of lesions or fluid Take photos of the heart 'in situ' (use scale marker as shown overleaf)
3	Examine the pericardium	Assess for lesions, thickening or fluid (if present quantify, characterise and sample). Formalin fix the pericardium.
4	Remove the pluck Remove heart from pluck	Check the anatomy of the great vessels before sectioning (especially in young animals) Use a needle and syringe to draw blood from the right atrium before opening and freeze the sample as whole blood (-80°C if possible, -20°C otherwise) Cut the pulmonary trunk transversely 3cm above the pulmonary valve – assess the lumen for thrombi Transect all vessels as far from the heart as possible
5	Examine the epicardium	Note any thickening, lesions, changes in appearance, colour etc. Wash/rinse the heart before taking photos of the heart from all sides
6	Open the ventricles	Make a single transverse incision through the lower third of the apex perpendicular to the long axis of the heart to expose the chamber of both ventricles– see explanatory image overleaf Remove any clots and rinse the heart before weighing
7	Weigh the heart	Record the weight (in grams)
8	Sample the apical myocardium	Take one 1x1x0.5cm portion of the sectioned piece of apex and place in a universal tube for freezing (at -80°C if possible, or -20°C otherwise) If RNA later is available, also preserve an additional portion of myocardium approx. 3x3x3mm in size and immerse in fluid
9	Fix the heart	Fully submerge the heart in 10% neutrally buffered formalin ensuring all surfaces are covered and there is sufficient formalin around the heart Leave to fix for at least 48 hours.
10	Perform gross post-mortem examination of rest of carcass	Open the entire aorta along itself length to the level of the iliac bifurcation; sample and formalin fix any lesions Examine the remaining major body organs as per the GATAG post-mortem protocol and take relevant samples for histopathology. Take special note of the lungs, liver and kidneys and where possible also provide a formalin fixed sample (1x1x1cm) of these
11	Complete paperwork	Complete the sample submission form
12	Contact us	Email heartproject@twycrosszoo.org
13	Send the heart	Refer to point 4 entitled "Sending Samples" above

Don't forget to take photos at all stages of the cardiac post-mortem examination (include a scale

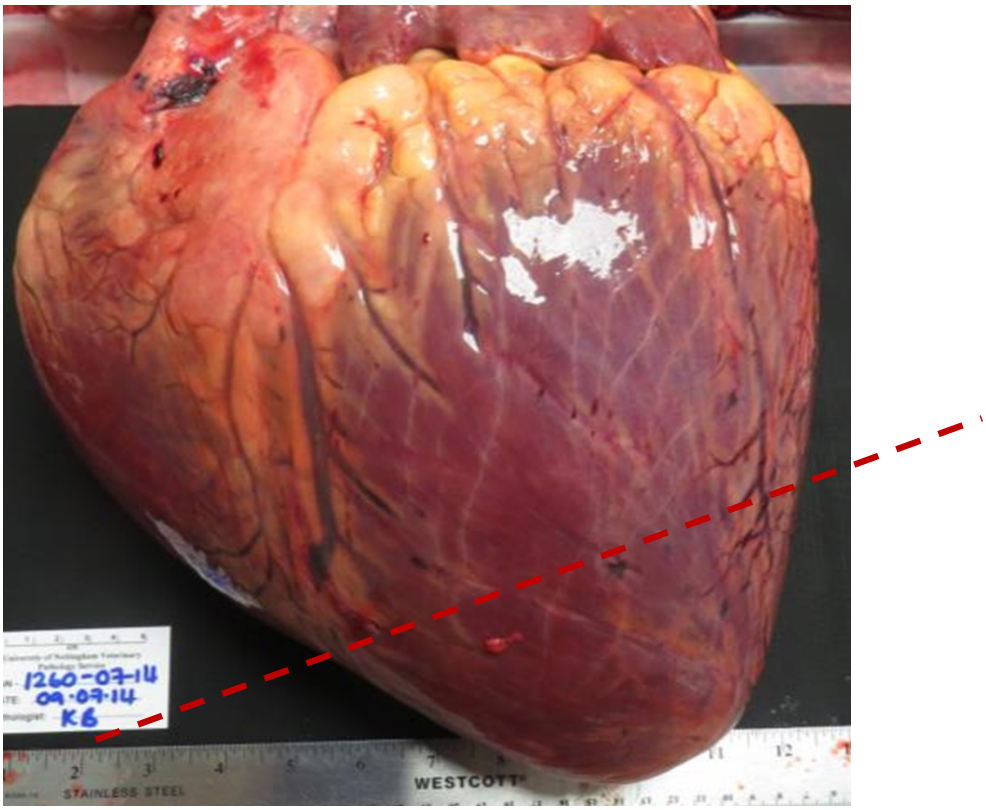
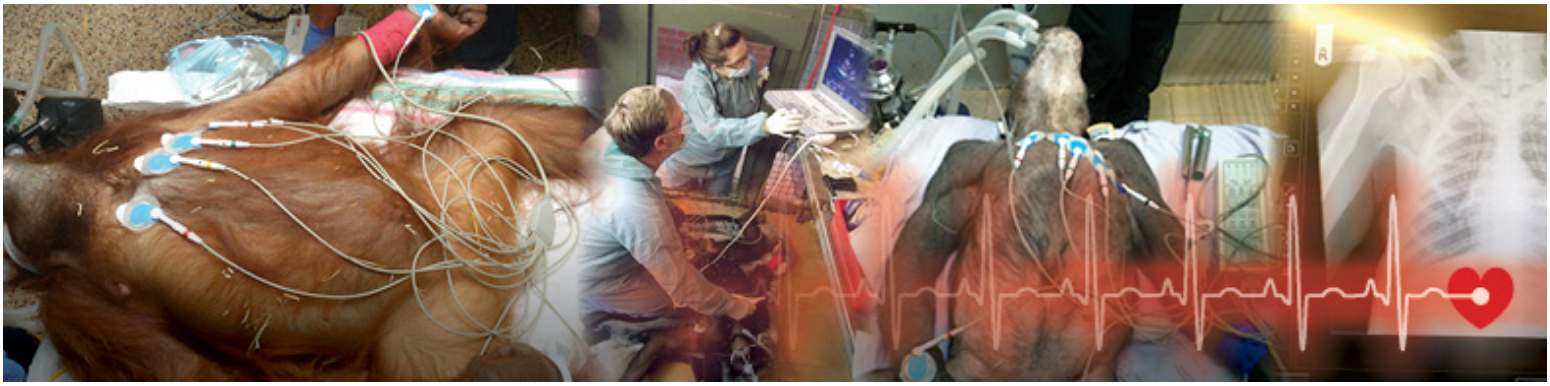


Figure 1: Shows the approximate location of the transverse cut to be made across the lower third of the apex. The cut should be made perpendicular to the long axis of the heart approximately 4cm from the apex in gorillas and 3cm in the other three great ape species. A single cut at this location should expose the chambers of both ventricles, allowing clots to be removed prior to weighing and fixing.

Don't forget to take photos at all stages of the cardiac post-mortem examination (include a scale

Appendix 11: Part 2 of the post-mortem protocol (detailed macroscopic and histopathologic examination)



GREAT APE CARDIAC PATHOLOGY:

Macroscopic examination and trimming protocol

Before starting the examination:

Information to gather:

- Animal ID: institutional ID (name, no.) +/- studbook number
- Taxa: chimp/bonobo/orang/gorilla
- Sex
- Age or DOB
- Institution/collection at the time of death
- Submitting institution (if different from above)
- Body weight
- Body condition score (out of 5)
- Date of death
- List of samples submitted
- PME findings
- History/circumstances of death
- Cause of death if known
- Heart weight (fresh)

Labelling system (to be used in EVERY case):

- e.g. C4 L1
- C = chimp; G = gorilla; B = bonobo; O = orangutan
- L = left ventricle
- R = right ventricle
- S = interventricular septum
- A = aorta
- SA = Sino atrial node
- AV = atrioventricular node
- 1 = anterior; 2 = posterior; 3 = lateral

What to set up:

- Safety cabinet
- Data collection form
- Scales
- Chopping board
- Large knife
- Trimming scalpel (handle and blade)
- Scissors
- String
- Pencil
- Cassettes (pre-labelled)
- Camera (memory card, battery etc.)
- Ruler

Process for examination:

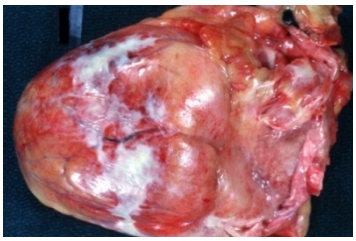
Conduct examination within safety cabinet. Record all findings/observations on data collection form

For all measurements, repeat three times to ensure consistency and accuracy If slightly different measurements are obtained each time, take a mean.

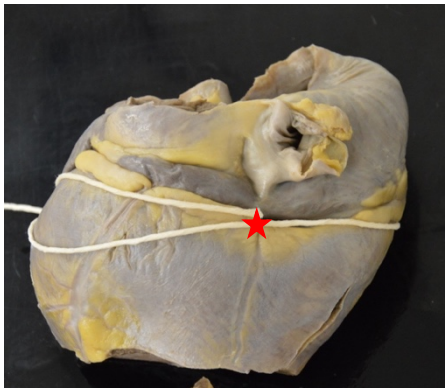
- Take photographs of:
  - All aspects of the heart: anterior, posterior, right lateral, left lateral, apex, base
  - Myocardial cross section
  - Any abnormalities/lesions detected or other findings described

- Comment on **condition of sample**
  - Has the sample already undergone extensive sectioning and trimming? If so does this limit extent of examination? (if so, comment)
  - Quality of tissue preservation? Fixing?
    - *Heart samples for which there was a delay between time of death and formalin fixation (during which time the sample might have been in cold storage) are generally be darker in appearance and can be friable to handle*
    - *Pink areas e.g. in the mid myocardium can suggest inadequate formalin penetration*
    - *Some mottling of the epicardium/myocardium can be seen (histopathology will distinguish these from lesions)*
  - Shape – does it appear to have been affected by fixing/transport?
    - Some hearts get squashed in transit, and heavy hearts (e.g. from gorillas) sometimes become slightly misshapen after sitting on the bottom of a container
  - Comment on amount of fat:
    - *It is normal to have fat in AV groove and extending along course of coronary arteries +/- covering the epicardium of the RV anterior and lateral walls (rarely the posterior wall).* Fat can also be associated with LV but never fully replaces LV wall in normal ageing heart
    - *In obese (human) patients the fat can cover the entire epicardial surface, and spread into the myocardium along course of intra-myocardial vessels (esp. RV and interatrial septum)*
    - *The wall needs to be fully replaced by fat, dilated and scarred to consider ARVC*

- Give a **morphological description**. Comment on:
  - Shape of heart
    - Is there any evidence of gross chamber enlargement? Any anatomical abnormalities?
  - Colour of the epicardium
  - Any lesions present on the epicardial surface
    - *Haemorrhages (can be seen on epicardium e.g. with CPR); infarcts; adhesions; ruptures?*
    - *In humans, white patches on the epicardium (called 'soldiers patches') are common (see image on right)*
      - *Particularly common on ant. wall of RV and apex of LV*
      - *Healed pericarditis? Mechanical trauma?*
      - *Histologically – fibrosis and lymphocytes*
      - *Of little clinical significance but should be noted*

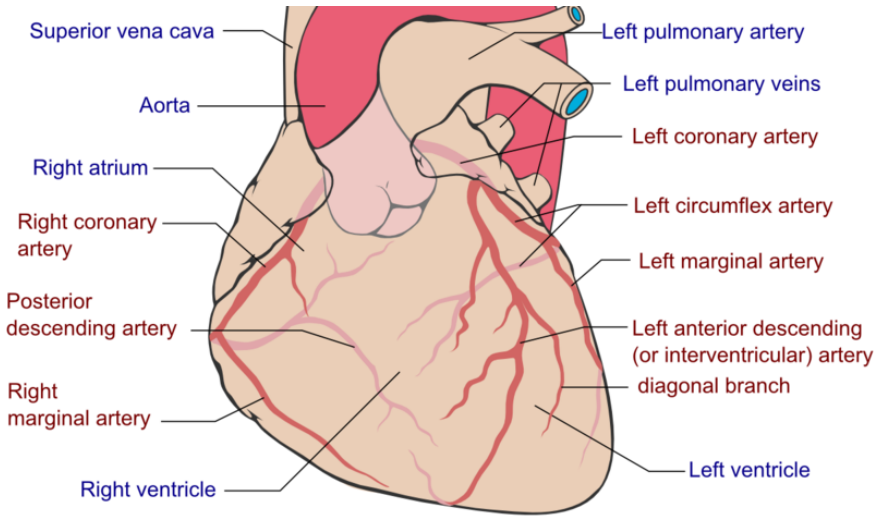


- **Weigh** the heart
  - Ensure all clots are removed before doing so (you may need to re-weigh later if clots are discovered as part of examination)
  - If significant portions of the heart are missing, make a note and do not weigh the heart

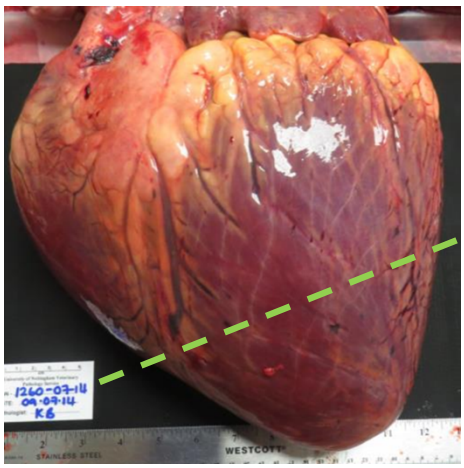


- **Take measurements** as follows:
  - Length: coronary/AV-groove, posterior aspect (see star, right) to tip of apex
  - Circumference at level of coronary groove (see right)
    - *Use string, and measure string against ruler*

- **Section the coronary vasculature**
  - A transverse cut across the aorta reveals cusps of aortic valve and coronary ostia
  - Probe the origins of both coronary arteries within the sinuses (probe 2-3mm)
  - Cut across coronary arteries at 3mm intervals (for nomenclature of coronary arteries, see diagram below
  - Assess for dilatation, atherosclerotic plaque formation

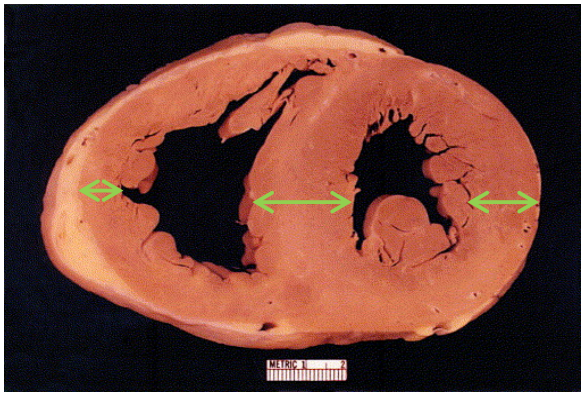


- **Make a transverse incision** across the lower third of the apex (see image, right). *This has usually been done by the submitting zoo as part of the protocol but the surface is often slightly irregular and often not perpendicular to the chambers, making transverse examination and measurements difficult/inaccurate. Making another cut slightly further up the heart, towards the base, improves this)*

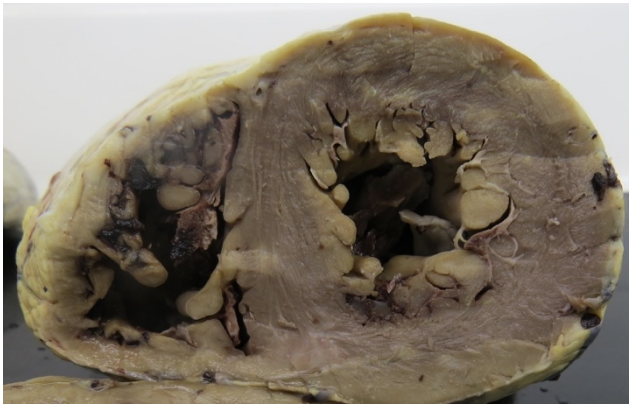


  - Measure:
    - LVFW
    - IVS
    - RVFW

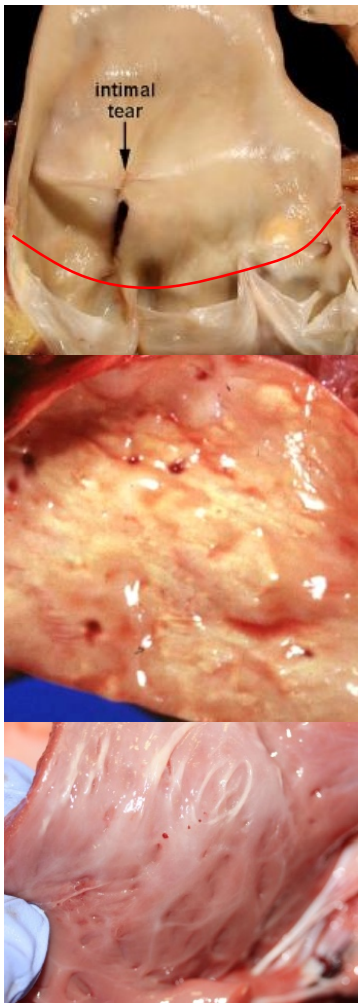
    Take care not to include papillary muscles or epicardial fat in these measurements (see diagram right)



- Assess the transverse myocardium for any evidence of mottling/infarction (to be continued later upon further transverse sectioning, following sampling for histopathology)
    - Note that is quite normal for there to be variation in colour of the myocardium due to differences in formalin update, for example: the papillary muscles, sub-endocardial +/- sub-epicardial myocardium are often paler in colour than the mid-myocardium (see image below). Make a note of this but state that it is probably artefactual.



- Open the **aorta:**
    - Assess for any lesions, for example:
        - *Thickened wall in aortitis*
        - *Entry tear in dissection (esp. first 3 cm)*
        - *Dilatation, aneurysm, intramural haematomas*
        - *Atherosclerosis (see image right, middle)*
        - Jet lesions *(see image right, bottom)*
    - Measure aortic valve circumference
        - *Use string and place it just above the valve cusps as shown by the red line on the top right image*



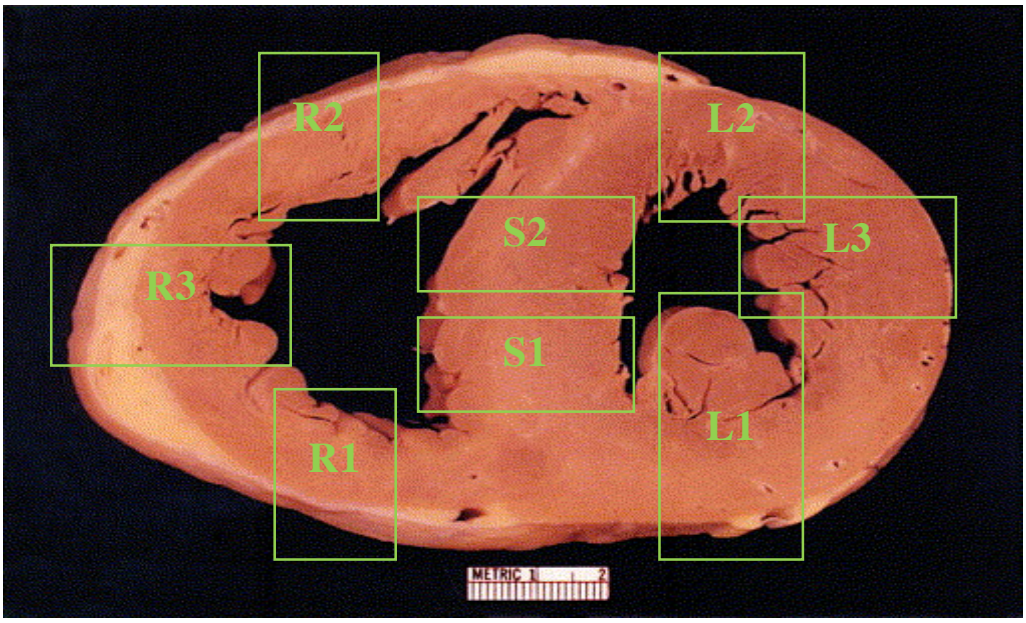
- Open **pulmonary artery:**
    - Assess for any lesions
    - Measure pulmonic valve circumference using the same technique as for the aortic valve

- Open **right atrium** (from inferior vena cava to RAA) **and ventricle** down lateral aspect
    - Measure the tricuspid valve circumference using the same technique as for the aortic valve
    - Check for ASD or PFO

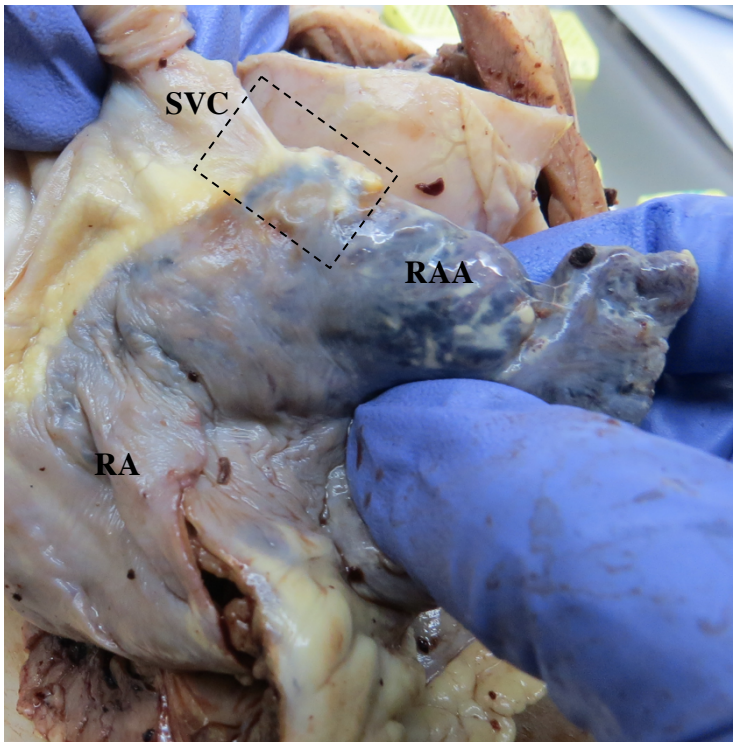
- Open **left atrium and ventricle** down lateral aspect
    - Measure the mitral valve circumference

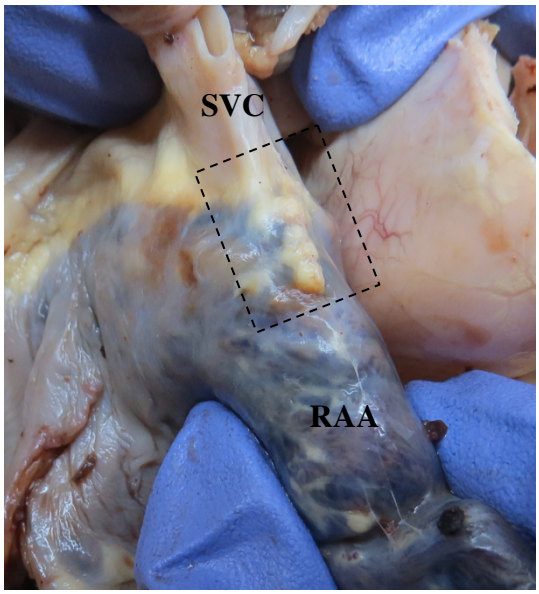
- **Inspect chambers** for abnormalities/lesions
  - In ARVC – fatty replacement of RVOT and area beneath tricuspid leaflet
  - Papillary muscles, MV and TV, chordae etc. – all normal?

- Take the following **samples for histopathology** and label each with the animal's study ID (e.g. C4) and location of sample (see overleaf). For further guidance on sampling locations, see image below or refer to Sheppard 2012.
  - Left ventricle: L1, L2, L3
  - Right ventricle: R1, R2, R3
  - Right ventricular outflow tract (below pulmonary valve): R4
  - Interventricular septum: S1, S2.
    - *Immerse the left side of the septum into blue dye and allow to dry in cassette before placing into formalin*

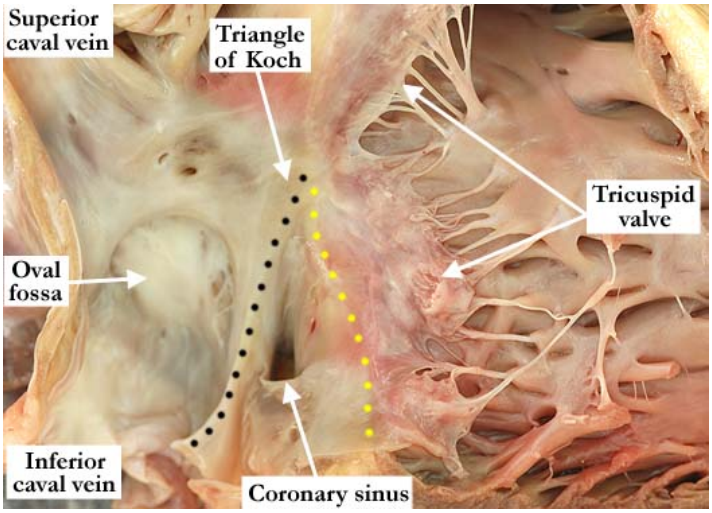


- Aorta: A
- SA node region of myocardium: SA
    - *SA node is sub-epicardial*
    - *Located in the groove at the junction of superior vena cava (SVC) and right atrial appendage (RAA) (see images below)*
    - *Take as a rectangular piece of tissue to include prox. superior VC and RAA wall*
    - *Place in cassette faced down*





- AV node region of myocardium: AV
  - *AV node is sub-endocardial*
  - *Atrial component lies in triangle of Koch (image, below), made up from:*
    - *Mouth of coronary sinus*
    - *Tricuspid valve*
    - *Membranous septum or oval fossa*
  - *Take as rectangular piece of tissue*
  - *Place in cassette faced down*

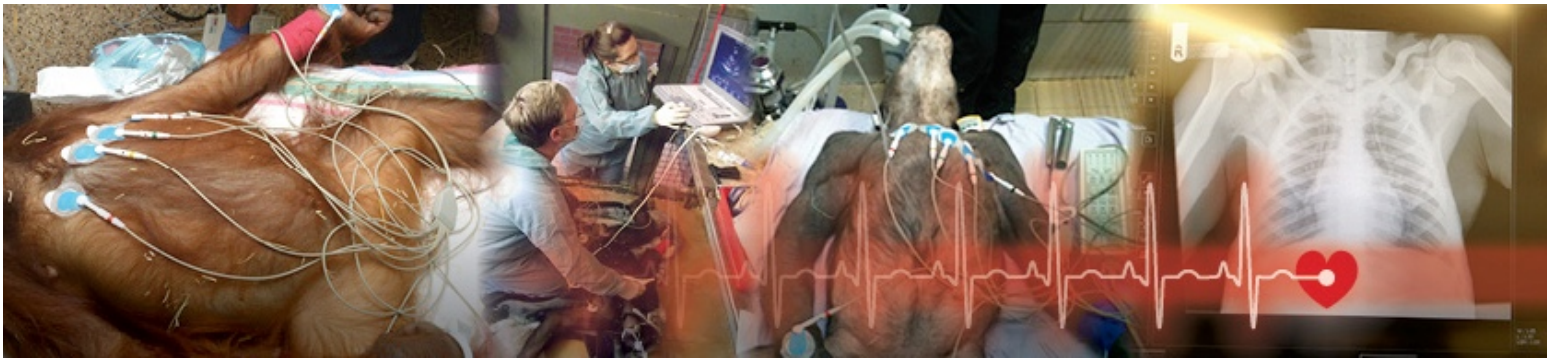


- Take additional samples of any abnormalities or lesions identified and label (make a note of label used)

- Once all samples have been taken (refer to checklist on data collection sheet), **make transverse incisions** (approx. 1-1.5cm intervals from apex to 2cm beneath AV groove) to examine all of the apical myocardium for infarcts/other lesions (bread loaf slicing technique)

- Submit for processing and histopathology preparation
  - H+E staining only as standard, additional stains can be requested if required

Appendix 12: Data collection form for post-mortem examination of heart



GREAT APE CARDIAC PATHOLOGY: DATA COLLECTION FORM

ANIMAL ID:	*Name:* *Study ID:*	**TAXA:**	
DOB: **AGE:**		**SEX:**	
BODYWEIGHT:		**COLLECTION:**	
DATE OF DEATH:		**DATE OF PME:**	
SAMPLES SUBMITTED:			
PHOTOS SUPPLIED?			
HISTORY/CIRCUMSTANCES OF DEATH:			
MAIN PME FINDINGS:			
HEART WEIGHT (fresh):			
CAUSE OF DEATH (if known):			

GROSS CARDIAC EXAMINATION FINDINGS (UoN):

DATE ARRIVED:		**TRANSPORTED BY:**	
STORED/HELD:			
DATE EXAMINED/ TRIMMED:		**TRIMMED BY:**	
STORAGE/TRANPORT COMMENTS:			
MORPHOLOGICAL DESCRIPTION:	***Sample quality:*** ***Overall/external appearance:*** ***Myocardial examination:*** ***Valves and vessels:***		

<table>
<tr><td>HEART WEIGHT (fixed):</td><td colspan="6"></td></tr>
<tr><td rowspan="8">MEASUREMENTS:</td><td colspan="3">Length (coronary groove to apex):</td><td colspan="3"></td></tr>
<tr><td colspan="3">CF (at coronary groove):</td><td colspan="3"></td></tr>
<tr><td colspan="3">LV : IVS : RV</td><td colspan="3"></td></tr>
<tr><td colspan="3">MV CF:</td><td colspan="3"></td></tr>
<tr><td colspan="3">TV CF:</td><td colspan="3"></td></tr>
<tr><td colspan="3">AV CF:</td><td colspan="3"></td></tr>
<tr><td colspan="3">PV CF:</td><td colspan="3"></td></tr>
<tr><td></td><td colspan="6"></td></tr>
<tr><td rowspan="7">SAMPLES TAKEN: (tick)</td><td>LV ✓</td><td>L1 (ant)</td><td>L2 (post)</td><td>L3 (lat)</td><td colspan="2">Other</td></tr>
<tr><td>RV</td><td>R1</td><td>R2</td><td>R3</td><td>R4</td><td>Other</td></tr>
<tr><td>IVS*</td><td>S1</td><td>S2</td><td colspan="3">Other</td></tr>
<tr><td>AV node</td><td colspan="5"></td></tr>
<tr><td>SA node</td><td colspan="5"></td></tr>
<tr><td>Aorta (A)</td><td colspan="5"></td></tr>
<tr><td></td><td colspan="5"></td></tr>
<tr><td>PHOTOS TAKEN:</td><td colspan="6">Yes/No</td></tr>
</table>

No. of samples submitted for histopathology:

* Denotes left ventricular endocardium was marked with blue ink

HISTOPATHOLOGICAL EXAMINATION FINDINGS (UoN):

EXAMINED BY:		**DATE OF EXAMINATION:**	
MAIN FINDINGS (per slide):	**L1**		
	L2		
	L3		
	L4		
	S1		
	S2		
	R1		
	R2		
	R3		
	R4		
	SA		
	AV		
	A		
	OTHER		

HISTOLOGICAL DIAGNOSIS(ES):	
COMMENT/ INTERPRETATION:	

Appendix 13: Table showing scoring system used for rating anaesthetic characteristics

Characteristic	Score	Description
Pre-anaesthetic activity level	**1**	None; very still
	2	Low; small amount of activity, e.g. moving to elsewhere in enclosure to avoid aim of dart gun
	3	Moderate; moving around enclosure to numerous locations prior to the successful administration of the induction agent
	4	High; constant moving around enclosure to numerous locations, resulting in significant prolongation of the time required to administer induction agent
Pre-anaesthetic demeanour	**1**	Depressed; collapsed, reduced or lack of awareness of external stimuli, e.g. sick or sedated animal
	2	Alert; aware of stimuli but reduced response compared with anticipated level of response, relaxed
	3	Apprehensive; responsive to stimuli, taking avoiding action e.g. hiding in corner of enclosure or behind another individual
	4	Aggressive; hyper-responsive to stimuli, displaying violent activity e.g. to the veterinarian administering induction agent
Quality of induction	***1***	Excellent; rapid, calm and smooth induction, recumbency and adequate and safe level of anaesthesia achieved
	2	Good; slightly prolonged but smooth induction, recumbency and adequate and safe level of anaesthesia achieved but some movement/response to stimulus remains e.g. able to be safely moved from enclosure but requires additional anaesthesia for intubation
	3	Fair; recumbency but not adequate/safe level of anaesthesia achieved, still has sluggish responses to stimuli and requires additional anaesthetic agents to be administered before can be safely handled
	4	Poor; inadequate sedative effect achieved, very responsive to stimuli, requires administration of additional anaesthetic agents before observers can enter enclosure
Degree of muscle relaxation	***1***	Excellent; trunk and limb muscle relaxed, no muscle tone or twitching
	2	Good; muscles relaxed, occasional mild muscle twitching in small focal regions of body, e.g. eyes, lips, hands, feet
	3	Fair; small amount of muscle tone, sustained or repeated muscle twitching of larger muscle bodies, e.g. whole limb movement
	4	Poor; muscles rigid, severe muscle twitching of multiple large muscle groups e.g. trunk and/or limbs
Depth of anaesthesia	***1***	Mild sedation; slight sedation, still able to move around without ataxia but at slower speed than usual
	2	Heavy sedation; responds to stimuli e.g. sound/touch with purposeful movements, safe level of anaesthesia for handling of e.g. non-dangerous animals
	3	Light anaesthesia; safe level of anaesthesia for handling dangerous animals, non-responsive to stimuli e.g. sound/touch, responsive to painful stimuli, most reflexes still present
	4	Surgical anaesthesia; non-responsive to painful stimuli, appropriate level of anaesthesia for e.g. surgical intervention, most reflexes are absent
	5	Excessively deep anaesthesia; excessive depression of central nervous system results in reduced ability to maintain normal functions e.g. respiration, animal at risk of anaesthetic death
Ease of intubation	***1***	Easy; coughing/gagging absent or minimal
	2	Moderate; small to moderate amount of reflex coughing/gagging but intubation achieved without need for additional (top-up) anaesthesia
	3	Difficult; pronounced coughing, intubation requires additional anaesthetic agents to be administered
	4	Extremely difficult; severe swallowing, coughing or gagging, intubation requires additional anaesthetic agents to be administered or is unsuccessful
Anaesthetic recovery	***1***	Excellent; smooth, non-vocal without paddling or uncoordinated movement
	2	Good; some minor paddling/excitation of short duration but no vocalisation or uncoordinated movement
	3	Fair; some vocalisation, paddling or uncoordinated movement but of short duration and easily calmed
	4	Poor; vocalisation, paddling or uncoordinated movement of moderate to severe duration and intensity

Appendix 14: Table showing individual animals' anaesthetic scores/ratings for both anaesthetic protocols (TZ & TZM)

Abbreviations: ND: no data collected/recorded; TZ: tiletamine-zolazepam administered for induction of anaesthesia; TZM: tiletamine-zolzepam and medetomidine administered for induction of anaesthesia.

Characteristic	Chimpanzee 1		Chimpanzee 2		Chimpanzee 3		Chimpanzee 4		Chimpanzee 5		Chimpanzee 6	
	TZ	***TZM***	***TZ***	***TZM***	***TZ***	***TZM***	***TZ***	***TZM***	***TZ***	***TZM***	***TZ***	***TZM***
Pre-anaesthetic activity level	3	4	4	4	3	2	4	4	ND	4	ND	4
Pre-anaesthetic demeanour	4	4	4	4	4	4	4	4	ND	4	ND	4
Induction quality	1	1	4	1	2	1	2	1	2	1	2	1
Ease of intubation	2	1	3	1	2	1	3	2	3	1	4	1
Muscle relaxation	1	1	2	1	2	1	3	1	2	1	3	1
Combined score	4	3	9	3	6	3	8	4	7	3	9	3
Depth of anaesthesia achieved	3	4	3	4	3	4	3	4	3	4	3	4

Appendix 15: Guidelines for performing a standard electrocardiogram (ECG) in great apes

Great Ape TAG - endorsed

A Guide to Performing a Standard Electrocardiogram (ECG) in Great Apes

Positioning

A 6 channel (basic; limb leads only) ECG can be performed with the animal in any position. Performing a 12 channel ECG, however, is aided by positioning the animal in dorsal recumbency.

Where possible, an electrocardiogram (ECG) should be performed routinely in all great ape anaesthetic procedures.

A **6 channel** ECG uses 4 electrodes placed on the limbs to gather basic information about heart rate and rhythm.

A **12 channel** ECG involves the placement of 6 additional electrodes on the chest. A 12 channel ECG provides a great deal more information about the electrical activity of the heart and can therefore aid in the diagnosis of more subtle changes such as myocardial ischemia or infarction.

Skin preparation:

- Clipping:
  - Adhesive electrodes can be placed on the dorsal or palmar/plantar aspect of the hands or feet, so no clipping is required
  - For the chest leads, it may be necessary to clip a small window if hair prevents good attachment of the electrodes
- The skin should be clean and clear of visible contamination. The application of surgical spirit to the skin can help to ensure good skin contact of electrodes

Electrode placement:

- Proper electrode placement is crucial in achieving an accurate and diagnostic ECG

Limb Leads:

LIMB	STANDARD	AMERICAN
Right fore	Red	White
Left fore	Yellow	Black
Left hind	Green	Red
Right hind (earth)	Black	Green

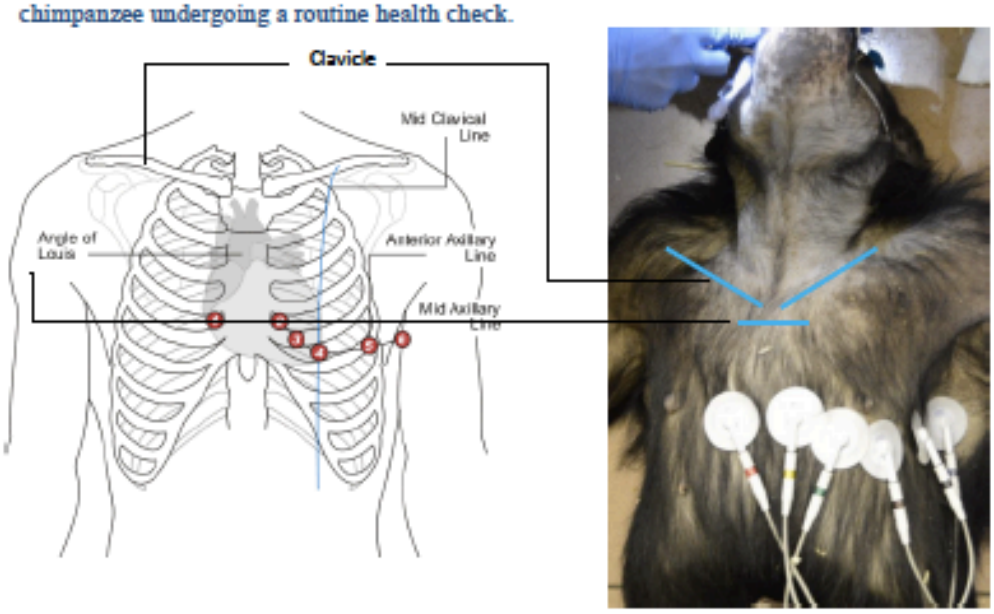
Table 1: Showing positioning and colour coding of ECG cables

Chest Leads:

LEAD	LOCATION	EXPLANATORY NOTE
V1	Fourth intercostal space (ICS) at right sternal border	Locate sternal angle (angle of Louis) – this is the 2nd rib Move to the right – this is the 2nd ICS. Move down 2 ribs to the 4th ICS Where this space meets the sternum is the position for lead V1
V2	Fourth intercostal space (ICS) at left sternal border	Go back to the sternal angle and move into the 2nd ICS on LHS Move down two ribs to the left 4th ICS Where this space meets the sternum is the position for lead V2
V3	Between fourth and fifth intercostal space (ICS)	Midway between V2 & V4 (Place V4 first)
V4	Fifth intercostal space (ICS), left mid-clavicular line	From the V2 position move down one rib to the 5th ICS (left) V4 is in this ICS in line with the middle of the clavicle
V5	Fifth intercostal space (ICS), left anterior-axillary line	Follow the 5th ICS to the left until your fingers are below the beginning of the axilla. This is the position for V5
V6	Fifth intercostal space (ICS), left mid-axillary line	Follow the 5th ICS further until you are immediately below the centre of the axilla. This is the position for V6

Figures 2a and 2b:

2a (left) shows the anatomical location of V1-6 chest leads[1]. 2b (right) shows ECG electrodes in situ on chimpanzee undergoing a routine health check.



Appendix 16: Research plan, submitted February 2015

Getting to the Heart of the Matter:
An investigation into captive great ape mortality and cardiovascular disease

Research Plan
Doctorate of Veterinary Medicine (DVM)
in zoo and exotic animal medicine

Supervisors:

Professor Malcolm Cobb, Kate White and Kerstin Baiker

School of Veterinary Medicine and Science, University of Nottingham

Sharon Redrobe and Sarah Chapman

Twycross Zoo, Burton Road, Atherstone, CV9 3PX

Contents

Abbreviations

BVZS	British Veterinary Zoological Society
CITES	Convention for International Trade of Endangered Species of flora and fauna
EAZA	European Association of Zoos and Aquaria
EAZWV	European Association of Zoo and Wildlife Veterinarians
EEP	European Endangered species Programme
ESB	European Studbook
(GA)TAG	(Great Ape) Taxon Advisory Group
ISIS	International Species Information System
SPARKS	Single Population Analysis and Records Keeping Software

1. Introduction: identifying the subject area

The term 'great ape' refers to the taxonomic family *Hominidae* which encompasses both the *Ponginae* and *Hominae* subfamilies and includes four great ape taxa; bonobo, chimpanzee, gorilla and orangutan[1]. Due to growing threats posed by the pet and bush-meat trades, habitat destruction and disease, wild populations of non-human great ape are in rapid decline and as a result, all species now feature on the IUCN Red List of Threatened species (IUCN, 2013).

As the wild population numbers of any species decline the role of captive animals increase in importance (Gusset et al. 2013). Captive great apes serve not only as ambassadors for their species in helping to raise funds and awareness but as a potential source for repopulation, thereby providing insurance against extinction. Due to their endangered status the international trade of all ape species is prohibited and the captive population must therefore be self-sustaining (CITES 2013). This relies heavily not only on the occurrence of successful births but the rearing of animals to sexual maturity and the maintenance of a population of healthy adults of sound reproductive status (Munson & Montali 1990). Any disease threat to captive apes is therefore of great concern not only for the individual or zoological collection affected but more widely, for the future breeding and conservation of these endangered and charismatic animals.

There are more than 1500 captive non-human great apes in zoological collections and sanctuaries across Europe (International Species Information System (ISIS) 2013). Many of those kept within zoos are managed as part of one of the European Association of Zoos and Aquaria (EAZA) breeding programmes; the European Studbook (ESB) or European Endangered species Programme (EEP). The EEP is the most intensive type of population management system within which a co-ordinator and species committee control matters such as animal moves and breeding. An EEP exists for the Western Chimpanzee, Bonobo, Western Lowland Gorilla and both Orangutan species. These EEPs along with the Common Chimpanzee ESB are assembled under the Great Ape Taxon Advisory Group (GATAG). The purpose of the TAG is to co-ordinate and advise upon regional collection planning, conservation, animal husbandry and where necessary, assist in matters of great ape health through the assigned veterinary advisors (EAZA 2013). The role of the veterinary advisor is to serve as a medical consultant on clinical cases and preventative health issues (EAZA 2011).

A number of reviews of diseases affecting great apes exist in the literature (Schmidt 1975; Cousins 1972; Benirschke & Adams 1980), the most recent of which was written by Janssen and Bush (1990). This paper reviews the medical literature of orangutans, gorillas and chimpanzees over the preceding decade. Janssen and Bush provide an overview of a number of conditions and conclude that reports of age related issues such as cardiovascular disease seemed to dominate the literature at this time. The husbandry and care of great apes in captivity is continually improving and as a result, patterns of morbidity and mortality changing. Given this fact it is important that individuals tasked with the custodianship of these animals, such as keeping staff and especially veterinarians, keep abreast of current and emerging trends in diseases. A systematic literature search for

[1]The *Ponginae* subfamily includes two species of orangutan; the Bornean (*Pongo pygmaeus*) and Sumatran (*Pongo abelii*). The subfamily *Hominae* encompasses three distinct genera; *Gorilla* (gorillas), *Pan* (chimpanzees) and *Homo* (humans). The non-human hominae are further divided into species; the chimpanzee (*Pan troglodytes),* bonobo (*Pan paniscus)* and the Eastern and Western gorilla species (*Gorilla beringei*, *Gorilla gorilla)* (Groves 2005).

publications on great ape morbidity and mortality since 1990 was performed and a number of key conclusions were drawn:

- There have been no reviews of the medical literature of great apes published since that by Janssen and Bush in 1990
- The majority of the literature comprises of reports of isolated clinical cases or outbreaks within one institution; there are only a handful of wider reviews in which attempts are made at quantifying the relative importance of various causes of morbidity or mortality
- Where wider reviews of morbidity/mortality have been performed, they are often restricted to certain species (mostly chimpanzee), countries (mostly the USA) and also frequently are from research facility rather than zoo populations
- The topic of cardiovascular disease in captive great apes is still very dominant within the literature
- Those reports on CV disease mostly concern American populations of gorillas and chimpanzees, the latter often being in laboratory rather than zoo settings
- Published papers on great ape mortality and also cardiovascular disease within the European captive population is generally lacking

It was therefore decided that the two main areas for the focus of this research would be:

- European captive (zoo) great ape overall mortality
- Cardiovascular disease as a cause of morbidity and mortality among the European captive great ape population

2. Project design and initiation

2.1 The current situation

There are a number of key areas identified as limiting progress with regards research in the area of great ape mortality and specifically cardiovascular disease, these have been articulated in the Great Ape Heart Project's White Paper (2012) and by the veterinary advisor for the European Great Ape Taxon Advisory Group (Sharon Redrobe – personal communication):

- There is ***no centralised database*** for the storage of information or samples. This means that there is a lack of data available for review or research, and also creates competition for resources.

- The veterinary care afforded to captive great apes in zoos across Europe varies dramatically between zoological institutions. This means that there is ***no standardised approach to;***

    - *Disease investigation/screening*
    - *Post-mortem examination*
    - *Sample collection and storage*

    This, in turn, results in a limited availability of data and samples, which are often of variable quality and consistency.

- Whilst the EEP co-ordinators and veterinary advisors do receive information about animal illnesses and deaths, utilisation of this system can be inconsistent (S. Redrobe, personal communication) and ***communication is limited***. This can limit knowledge and awareness of problems at the EEP level, and also means that use of available expertise is not always maximised.

- There is a ***lack of co-ordination*** of research and investigative efforts. This leads to a non-targeted approach, can create competition for resources and has the potential to lead to repetition of work.

Review of the current situation therefore suggests a need for a Europe-wide initiative aimed at improving understanding of all diseases affecting captive great apes. The development of this initiative is valuable in ensuring the success of this particular research project, but also has wider ramifications in aiding the progression of future research in this area.

This initiative will aim to:

- Improve communication between zoo staff, vets and interested personnel with relevant expertise
- Lead to the creation of a database/bank for mortality data, clinical information and relevant samples
- Standardise the approach to post-mortem examination, disease investigation and screening and sample collection and storage.
- Co-ordinate research and promote collaborative approach to investigation

The stages anticipated to be involved in this are outlined in sections 2.2-2.5 below:

2.2 Recruiting personnel

The success of such a widespread initiative is dependent on the collaboration of a team of individuals. The first stage will therefore involve the recruitment of interested personnel from a variety of areas, including vets, cardiologists, echocardiographers, pathologists, epidemiologists, anaesthetists and animal training staff. The recruitment will mostly involve targeted contact of specific individuals and both email and face-to-face communication.

2.3 Obtaining approval

Ethical approval will be obtained prior to the commencement of research. This will be sought through the University of Nottingham School of Veterinary Medicine ethical review committee. The project will also be run as an EAZA GATAG approved project, which will further enhance compliance; all proposals and protocols will therefore be submitted to the EAZA GATAG for approval prior to use/dissemination.

2.4 Producing documentation and protocols

A number of documents need to be produced for initiation of the study:

- *Letters; t*o be sent to all great ape holding institutions under study requesting their involvement and assistance
- *Protocols;* e.g. for post-mortem examination, sample collection and storage, and disease screening.
- *Forms;* e.g. data and sample submission forms

Each of these documents must be granted approval prior to use/dissemination. This will be achieved by communication with the EAZA GATAG committee and research committee, through the vet advisor Sharon Redrobe.

2.5 Raising awareness

The success of this initiative is dependent on awareness of the project and support for it among the zoo and veterinary communities. The project will be promoted and awareness raised by the following means:

2.5.1 Production of a webpage dedicated to the project;

- To contain introductory information about the project purpose and aims
- To act as a resource for vets and cardiologists; forms and protocols will be freely accessible

2.5.2 Presentation at conferences and meetings;

- EAZA Congress
- British Veterinary Zoological Society (BVZS) meetings
- Continued professional development courses and sessions
- The European Association of Zoo and Wildlife Veterinarians (EAZWV) congress

2.5.3 Dissemination of information in the literature

- Letters and articles for example in;
    - The Veterinary Record
    - The Veterinary Times
    - EAZWV newsletter
    - Zoo vet mailing lists

2.5.4 Contacting/targeting key groups;

- Those zoos across Europe which house the most great apes will be identified using the International Species Information System (ISIS)
- These zoos will be contacted directly to inform them about the project and to offer them an information pack +/- sampling kit

3. Epidemiological Study

Epidemiology is the study of how often diseases occur in different groups of individuals and why. This, alongside the clinical findings and pathology, forms an integral part of the basic description of a disease. This section of the research study will involve a widespread study of great ape mortality and then more specifically the epidemiology of great ape cardiovascular disease. This study will be separated into two main parts:

3.1 Mortality Review

A systematic review of literature relating to great ape morbidity and mortality published between 1990-2014 was performed (Strong, 2014, DVM thesis). One of the key conclusions drawn was the need for an up to date, widespread review of mortality within the European captive great ape population.

For this review, the population under study will be those great apes that are managed as part of the EAZA EEPs or ESBs.

3.1.1 Protocol for data collection:

For each of the four great ape taxa, a list of all deaths that have occurred since 1st January 2004 will be generated from the studbook data (SPARKS report). Each of the zoos will be contacted via letter/email (co-signed by the great ape TAG veterinary advisor and the relevant species co-ordinator). For each death, a copy of each of the following will be requested:

- Post-mortem report
- Animal records
- Clinical history/medical records

For each death, the zoo will also be asked to provide information on what (if any) in-vivo and post-mortem samples they have available (for example - urine, blood, heart tissue). The raw data will be reviewed and the following information gathered and entered into a database (N.B. all four species of great ape will be dealt with separately)

- Animal identification (regional studbook number, name and study identification code)
    - The study identification code will be unique to that animal. It will permit traceability whilst allowing confidentiality to be maintained in data presentation.
- Zoological collection (holding zoo at the time of death):
    - For reference only - this will be excluded from data presentation in order to maintain confidentiality.

- Subspecies (if known)
- Sex (male, female or unknown)
- Date of birth and date of death
    - Used to calculate the age at death (For calculation purposes - where only a month and year is provided, the date of birth will be denoted as the 1st of the month. Where only a year is provided, the date of birth will be denoted as 1st July of that year)
    - The age at death will then be categorised (age limits for each category will vary by species), e.g. abortion/premature/stillborn, neonate, infant, juvenile, adult, aged adult
- Post-mortem examination performed: yes/no
- Diagnosis/cause of death determined: yes/no
- Nature of death (i.e. natural vs. euthanasia)
- Circumstance of death (for example; perinatal, peri-anaesthetic, following illness, sudden death, trauma, unclassified)
- Cause of death: Free text and categorised (the latter being coded according to aetiology and body system affected)
- Available samples (free text field)

The information obtained will be reviewed and checked for data input error. Numerical data will be subject to appropriate quantitative statistical analysis, dependent on the size of the dataset obtained. Qualitative data will be reviewed and thematic analysis performed to identify trends in the dataset.

By using the methods outlined above, the following key questions will be answered;

- With what frequency are post-mortem examinations performed in zoological collections across Europe?
- With what frequency is a diagnosis/cause of death determined?
- What is the average life expectancy for each taxa?
- For each taxa, what is the rate of a) perinatal mortality and b) infant mortality?
- What proportion of animals are euthanased versus die naturally?
- What are the most common causes of death;
    - Categorised according to aetiology
    - Categorised according to body system
    - In each taxa
    - In each age category
    - For each sex
- What is the most common circumstance of death?
- Specifically, what proportion of deaths are caused by cardiovascular disease;
    - In each taxa

- In each age category
- For each sex

3.2 Cardiovascular disease review and risk factor analysis

3.2.1 Cardiovascular disease review

The taxa in which cardiovascular disease is most significant, as identified in Part 1, will then be the subject of subsequent investigation.

3.2.1.1 For these animals the original data will be further scrutinised and additional questionnaires disseminated in order to gather more information as outlined below:

- Origin (wild, captive, unknown)
- Parentage (regional studbook number of the dam and sire)
- Rearing history (parent, hand-reared, part hand-reared, unknown)
- Contraceptive status (male -none, surgical, unknown; female - none, surgical, hormonal implant, oral contraception, unknown)
- Body mass/body condition (body condition score, crown rump length, body weight, body mass index)
- Group information
  - Group number (continuous variable; categorised for analysis)
  - Group 'type' (single sex, mixed sex, family)
  - Social position within group
  - Sex ratio of group
- Time elapsed since last health assessment:
  - 0-7 days, 8-28 days, 1-6 months, 6-12 months, 13-24 months, >24 months
- Whether the animal received cardiac assessment in life, and if so whether copies of the findings/original data are available (yes/no)
- Whether or not that animal showed any of the recognised symptoms of cardiovascular disease (cough, weakness, collapse etc.)
- The number of the following the animal had undergone in the 6 months preceding death;
  - General anaesthetics (for elective procedure, for example move or health check)
  - General anaesthetic for unplanned procedure (for example surgery or medical treatment)
  - Group changes (the individual itself)
- The number of the following events that occurred within the animal's group in 18 month period preceding death? (Continuous variable; categorised for analysis)
  - Deaths
  - Births
  - Introductions (into the group)
  - Relocations (out of the group)

- Information regarding other possible risk factors including:
  - Diet (content, way it is fed)
  - Enrichment (type, frequency)
  - Enclosure design (encourage activity, climbing etc., access to 'off show' areas)
  - Infectious disease status of individual and group (previous serology results)

The data listed above will be added to the database. Qualitative data will be reviewed and thematic analysis performed to identify trends in the dataset. Numerical data will be subject to appropriate quantitative statistical analysis, dependent on the size of the dataset obtained.

By the methods outlined above, the following key questions will be answered:

- Which cardiovascular pathologies were reported/diagnosed?
- How many animals underwent cardiac assessment, and what clinical data is available?
- With which circumstances of death is cardiovascular disease most often associated? (i.e. following illness versus sudden versus peri-anaesthetic)
- What symptoms did affected animals show?
- Is the presence of symptoms (cough, weakness etc.) predictive for death due to cardiovascular disease?

3.2.2 Risk factor analysis

The data collected above will be subject to risk factor analysis to assess for the degree of association between death due to cardiovascular disease and each of the following variables:

Age, origin, zoological collection, subspecies, parentage, sex contraceptive status, body mass/condition, group number, group type/composition, social status, time since last health assessment, recent anaesthesia, recent group changes etc.

For any associations identified, the statistical significance of the association will be calculated. The data will be assessed for the presence of any confounding variables which may skew the findings.

3.3 Proposed outcomes

- Identify the main causes of mortality across each of the four great ape taxa
- Compare patterns of mortality between animals of differing species, age and gender
- Identify the main causes of a) sudden/unexpected death and b) peri-anaesthetic death
- Ascertain the significance of cardiovascular disease as a cause of death in each of the great ape taxa

- To have studied the role that each of the above factors may have in the risk of developing or dying from cardiovascular disease
- To be aware of the number and types of samples available for further research

4. Clinical investigation of cardiovascular disease

As previously stated there is currently no standardised approach to the clinical investigation of, or screening for cardiovascular disease among great apes in captivity across Europe. A number of zoos are performing assessments in the form of echocardiography, ECG and/or measurement of circulating cardiac biomarkers. However, there appears to be much variability in the tools being used, the qualifications/skill level of those performing the assessments, the echocardiographic views being obtained and the measurements being taken. There is also very little known about what is 'normal' for these animals, making interpretation of the findings very difficult.

This of course, does not invalidate such assessments; from an individual animal standpoint, such assessment is sufficient for basic clinical utility. However, from a research point of view, it makes comparison between findings from multiple individuals, or the same individuals at different time points difficult; it also limits our ability to form conclusions/generalisations from large amounts of data or to make comparisons between them. This highlights the need for a standardised approach.

4.1 Creation of a cardiac assessment protocol for great apes

A protocol for the cardiac assessment of great apes will be produced; it will consist of guidelines for performing echocardiography, ECG, blood pressure and cardiac biomarker assessment. The stages involved in creation of the protocols are outlined below:

- Identifying the aims of the protocol and the key areas to be covered
- Studying similar protocols for other species, including humans
- Creation of a draft protocol; this will require the experience and expertise of others within the team
- In-house implementation of the protocol: pilot use of the protocol in-house at Twycross Zoo will allow the user friendliness and quality of the protocol to be assessed and amendments to made as necessary
- Assessment of reliability and reproducibility of data collection: repeated data collecting using the protocol will be performed and successive datasets assessed for consistency and repeatability

- Publication and awareness: once all of the above steps are complete, the protocol will be released for use and disseminated by the methods discussed previously (conference attendance, veterinary press, website etc.)

4.2 Data collection and analysis

The information gathered from each clinical assessment will be reviewed and relevant data extracted for input into a database. Qualitative data will be reviewed and will be subject to thematic analysis. Numerical data will be assessed for normality before being subjective to appropriate statistical analysis which will be determined by the size of the dataset.

4.3 Proposed outcomes

- To understand more about the utilisation of clinical cardiac assessment and indeed specific diagnostic modalities, and their application in detecting great ape cardiovascular disease
- To have an appreciation for which pathologies and/or conditions can be/have been diagnosed ante-mortem using clinical cardiac assessment
- To have a greater understanding of the "clinical picture" of a great ape with cardiovascular disease (i.e. clinical signs, presentation etc.)
- To study the benefits or potential benefits of early detection of disease
- To have an appreciation for what is "normal" in each of the species under study
- To have considered, and potentially studied, the effects of anaesthesia on the accuracy and therefore the clinical application of these diagnostic modalities

5. Detailed study of great ape cardiovascular pathology

Whilst clinical screening for ante-mortem detection of cardiovascular disease in great apes is of major importance, our current ability to do so is limited by our incomplete understanding about the underlying aetiopathogenesis. There is currently no standardised approach to the post-mortem examination of the cardiovascular system or sample collection for histopathology between different collections across Europe. This means that diagnoses are likely to be missed and the reporting of cardiovascular system changes and abnormalities are inconsistent, and where reporting is performed the terminology applied is inconsistent. A review/study of cardiovascular pathology will therefore be performed, split into two main parts:

Where possible, archived tissues or histopathology slides will also be sought and the histopathology reviewed under supervision of a European-boarded veterinary pathologist. A systematic process will be developed for reviewing consistent tissue areas and uniform terminology according to medical and veterinary pathology standards will be applied to the findings.

5.1 Retrospective study

For those great ape deaths identified in the mortality review, the post mortem examination records will be sought. The records will be reviewed and a summary of the contents input into the database.

Where possible, archived tissue sections or histopathology slides will also be sought and the histopathology reviewed under the supervision of a European boarded pathologist.A systematic process will be developed for reviewing consistent tissue areas and uniform terminology according to medical and veterinary pathology standards will be applied to the findings.

Thematic analysis will be performed on the resultant pathological descriptions in order to identify trends in cardiac pathologies/disorders identified among the population under study.

5.2 Prospective study

A standardised protocol for macroscopic examination and sampling of the cardiovascular system will be created, with the assistance of others within the team. The protocol will be disseminated to zoos, zoo vets and veterinary pathologists across Europe. EAZA member zoos will be asked to encourage their vets/pathologists to follow this protocol during post-mortem examination, to sample and fix the heart as per project recommendations, and to send the heart to the project for in depth examination.

For all heart samples that are collected, additional biological samples such as whole blood and RNA-later fixed heart tissue will also be stored in the University of Nottingham School of Veterinary Medicine tissue biobank; this will permit future molecular work linking the genotype and phenotype of affected individuals.

A more detailed protocol for microscopic examination of heart samples will be created, based upon gold standard detailed cardiac examination protocols developed for use in human medicine. This protocol will be used and all available hearts examined. It will specify multiple relevant functional areas which have to be consistently sampled and processed for routine histology and special stains. A systematic process will be followed and consistent terminology applied to describe the findings. The data collected will be subject to thematic analysis in order to identify the common pathological changes occurring in the cardiovascular system of captive great apes

5.3 Proposed outcomes

- To encourage a systematic approach to the post-mortem examination of the cardiovascular system in order to maximise diagnosis and enhance understanding of great ape cardiovascular disease
- To standardise the terminology used for cardiovascular pathologies observed in great apes
- To identify;
    - What disorders are affecting the cardiovascular system of great apes?
    - How do these conditions appear; grossly and histologically?
    - What pathology characterises each ‘condition’
- To permit future research and comparison between reported generated and samples gathered by various individuals, at differing institutions on numerous occasions

Appendix 17: Published paper: A systematic review of the literature relating to captive great ape morbidity and mortality. Journal of zoo and wildlife medicine. 2016 Sept; 57 (3): 697-710

Journal of Zoo and Wildlife Medicine 47(3): 697–710, 2016

A SYSTEMATIC REVIEW OF THE LITERATURE RELATING TO CAPTIVE GREAT APE MORBIDITY AND MORTALITY

Victoria J. Strong, B.V.Sc., B.Sc. (Hons) MRCVS., Douglas Grindlay, Ph.D., M.C.L.I.P., Sharon Redrobe, B.Vet.Med., D.ZooMed MRCVS, Malcolm Cobb, D.V.C., Ph.D. MRCVS, and Kate White, Vet MB Dipl. E.C.V.A.A. MRCVS

Abstract: Wild bonobos (*Pan paniscus*), chimpanzees (*Pan troglodytes*), Western gorillas (*Gorilla gorilla*), and orangutans (*Pongo pygmaeus, Pongo abelii*) are threatened with extinction. In order to help maintain a self-sustaining zoo population, clinicians require a sound understanding of the diseases with which they might be presented. To provide an up-to-date perspective on great ape morbidity and mortality, a systematic review of the zoological and veterinary literature of great apes from 1990 to 2014 was conducted. This is the first review of the great ape literature published since 1990 and the first-ever systematic literature review of great ape morbidity and mortality. The following databases were searched for relevant articles: CAB Abstracts, Web of Science Core Collection, BIOSIS Citation Index, BIOSIS Previews, Current Contents Connect, Data Citation Index, Derwent Innovations Index, MEDLINE, SciELO Citation Index, and Zoological Record. A total of 189 articles reporting on the causes of morbidity and mortality among captive great apes were selected and divided into comparative morbidity–mortality studies and case reports–series or single-disease prevalence studies. The content and main findings of the morbidity-mortality studies were reviewed and the main limitations identified. The case reports-case series and single-disease prevalence studies were categorized and coded according to taxa, etiology, and body system. Subsequent analysis allowed the amount of literature coverage afforded to each category to be calculated and the main diseases and disorders reported within the literature to be identified. This review concludes that reports of idiopathic and infectious diseases along with disorders of the cardiovascular, respiratory, and gastrointestinal body systems were particularly prominent within the great ape literature during 1990–2014. However, recent and accurate prevalence figures are lacking and there are flaws in those reviews that do exist. There is therefore a critical need for a robust, widespread, and more up-to-date review of mortality among captive great apes.

Keywords: Bonobo, chimpanzee, gorilla, mortality review, orangutan, zoologic.

INTRODUCTION

As a result of the growing threats posed by the pet and bush-meat trades, habitat destruction and disease, wild populations of all great apes (bonobos, chimpanzees, gorillas, and orangutans) are rapidly diminishing.[56] Therefore great apes housed in zoos serve not only as ambassadors for their species in helping to raise funds and public awareness of conservation issues but as a potential source for repopulation, thereby providing insurance against extinction. Due to their endangered status, the international trade of all ape species is prohibited although movements between zoos is permitted for breeding pruposes.[29] The zoo population must therefore be self-sustaining and the management of the breeding programs is guided within Europe by the Great Ape Taxonomic Advisory Group (TAG) under the European Association of Zoos and Aquaria. The success of the program relies on the occurrence of successful births, the rearing of animals to sexual maturity, the maintenance of a population of healthy adults of sound reproductive status and the genetic management of the population to maintain genetic diversity and health.[90] Any disease threat to captive great apes is therefore of great concern not only for the individual or zoologic collection affected, but more widely, for the future breeding and conservation of these species.

Historically, infectious diseases and disorders of the gastrointestinal and respiratory systems have been consistently identified as the most significant causes of morbidity and mortality among captive great apes.[9,32,112] However, as a consequence of advances in animal husbandry and veterinary care, patterns of disease are continually changing. Janssen and Bush[59] published an overview of a number of diseases that had been reported to affect orangutans, gorillas,

From the School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, United Kingdom (Strong, Grindlay, Cobb, White); and Twycross Zoo, Burton Road, Atherstone, Warwickshire, CV9 3PX, United Kingdom (Strong, Redrobe). Correspondence should be directed to Dr. Strong (ntxvjs@nottingham.ac.uk).

and chimpanzees during the 1980s. One of their conclusions was that age-related conditions such as gonadal neoplasia and cardiovascular disease dominated the literature at this time. There are, however, two potential criticisms of this review. Firstly, with the exception of the aforementioned statement about cardiovascular and neoplastic disorders, there was little attempt to quantify the relative importance of each condition. Also, given that the paper was a narrative review with no methodology provided, it is difficult to confirm the completeness of the information presented.

To the authors' knowledge, there have been no reviews of the veterinary literature of great apes published since 1990. With a view to expanding upon the work of Janssen and Bush,[58] a systematic review of the zoologic and veterinary literature of great apes from 1990 to 2014 was conducted. The aims of the study were as follows: 1) to identify and critique papers that report upon the comparative prevalence of various diseases and disorders (morbidity and mortality reviews) within the captive great ape population and 2) to calculate the amount of literature coverage afforded to each of the various causes of great ape morbidity or mortality in the zoologic and veterinary literature between 1990 and 2014 by taxa, etiology, and body system affected.

METHODS

Methodology for literature search

The methodology for this systematic literature review followed published guidelines.[21,100]

Eligibility criteria

The research question was defined as the following: "What are the main causes of morbidity and mortality among great apes kept in captivity?" The inclusion criteria were set to include papers that reported upon specific, naturally occurring diseases or disorders of clinical relevance among captive bonobos, chimpanzees, gorillas, and orangutans.

Information sources

Databases were selected based upon their reputation, relevance, and reported coverage of the literature.[45] CAB Abstracts is reported to be the database that provides the widest coverage of veterinary journals and was selected for this reason. Also selected for their breadth of coverage were databases available through the Web of Knowledge interface covering a wide range of topic areas: Web of Science Core Collection, BIOSIS Citation Index, BIOSIS Previews, Current Contents Connect, Data Citation Index, Derwent Innovations Index, MEDLINE, and SciELO Citation Index. Zoological Record was searched due to its coverage of zoology-specific journals not indexed elsewhere.

Search

Alternative search terms and synonyms for each of the key words within the research question were used. The search was limited to include only those papers in English and those published since 1990. The papers obtained from all three databases were merged and duplicates removed.

Article selection and sorting

Papers were screened for relevance and the final list of relevant publications was exported to an Excel spreadsheet.[84] The content of each was reviewed and the papers classified as either 1) comparative morbidity-mortality studies or 2) case reports–series and papers reporting upon the prevalence of single diseases.

Data collection and processing

The morbidity-mortality studies were reviewed and the following data extracted from each: author, date of publication, period of study, type of study (retrospective-prospective, interventional-observational, multi-single center, longitudinal or cross-sectional), details relating to the study population (taxa, number of subjects, age, sex), and the main causes of morbidity-mortality identified. The reviews were assessed for risk of bias and the main limitations of each identified.

The contents of the case reports–case series and single-disease frequency studies were reviewed and each article categorized and coded according to 1) the taxa under study, 2) the etiology of the primary condition being reported, and 3) the body system affected. Etiologic categories were adapted from the DAMNITV classification system as used by Rizzo et al.[106] and on categories used by Mesle.[83] Body system categories were adapted from those used by Robinson et al.[107] Descriptive statistics were carried out and the amount of literature coverage afforded to each of the categories was calculated. The main diseases and disorders reported within the literature were also identified.

RESULTS AND DISCUSSION

The initial search yielded 1,146 results and following relevance screening, 189 full-text arti-

FIGURES

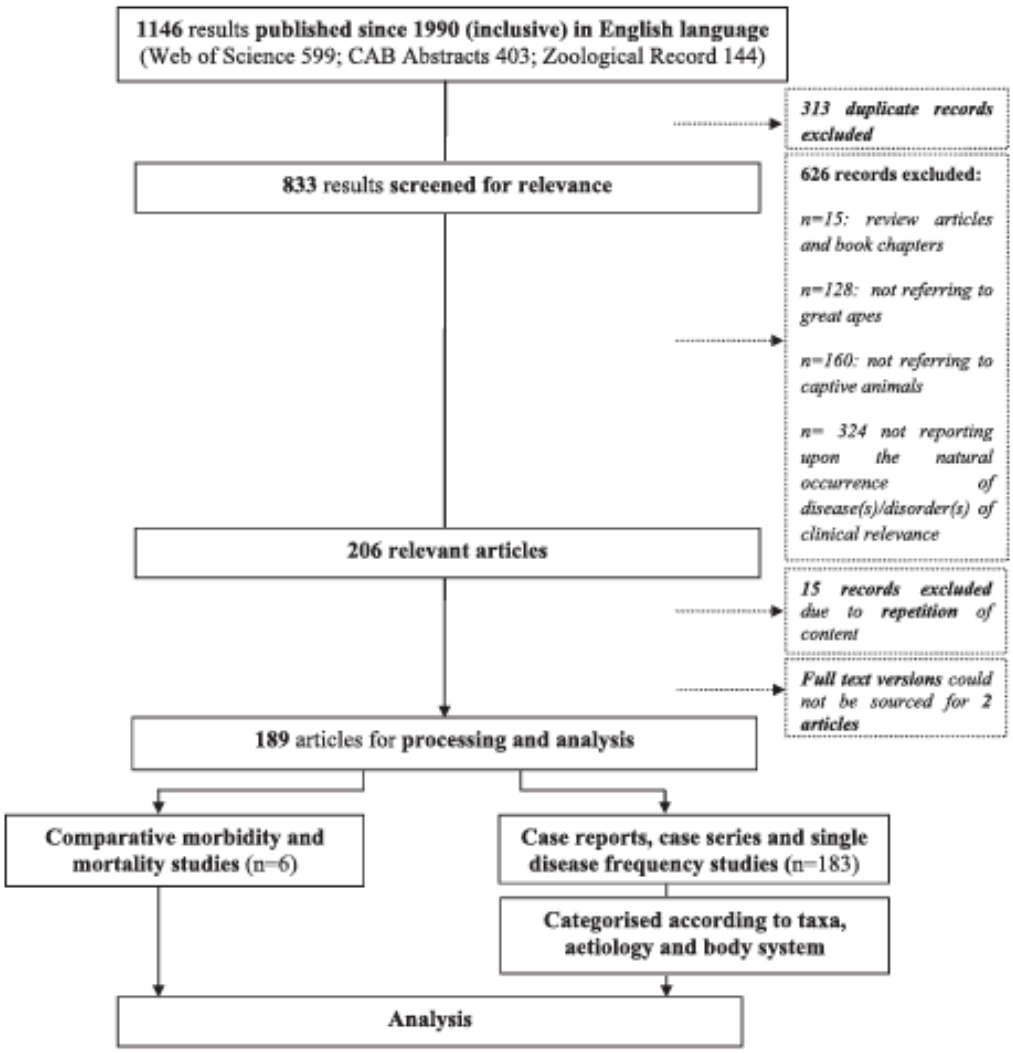


Figure 1. Flow chart showing sequential steps involved in reference selection and exclusion process based on the PRISMA checklist and flow diagram.

cles remained (Fig. 1). The literature search revealed a variety of reference types: journal articles, conference proceedings, meeting papers, and correspondence pieces. A total of 183 (97%) of the articles were classified as case reports, case series, and single-disease frequency studies. The remaining six papers were morbidity and mortality studies, which reported upon the comparative frequency of occurrence of various diseases and disorders within a population.

Case reports, case series, and single-disease frequency studies

The absolute number and percentage of papers identified for each taxon are displayed in Table 1. Chimpanzees (40%) and gorillas (35%) received greater literature coverage than orangutans (19%) and especially bonobos (2%). This distribution may be related to the relative numbers of each species kept in captivity and due to the involve-

Table 1. Amount of literature coverage afforded to each taxa, displayed as absolute number and percentage of total ($n = 1,831$), ordered alphabetically.

Taxa	Absolute number	% of total
Bonobos	4	2%
Chimpanzees	73	40%
Gorillas	64	35%
Orangutans	35	19%
All four taxa	2	1%
Two or three taxa	5	3%

ment of chimpanzees in biomedical and behavioral research.

Etiology: A total of 174 (95%) of the 183 papers could be categorized according to the etiology of the primary condition being reported (Table 2). The three etiologies that received most literature coverage were infectious (39%), idiopathic (17%), and neoplastic (9%) disorders.

Infectious diseases, especially of the gastrointestinal and upper respiratory tracts, were reported more commonly in the literature from 1990 to 2014 than disorders of any other etiology. This is despite the fact that infection has been stated elsewhere as becoming less important over recent years.[130] The papers that reported upon infectious disease were further categorized by causal agent (Table 3). Bacterial infection was often reported in association with disorders of the gastrointestinal system such as enteritis, colitis, and the clinical presentation of diarrhea. Reported pathogenic agents include *Salmonella* spp., *Escherichia coli* and *Campylobacter* spp.[10,95,97] The most commonly cited gastrointestinal disorder of bacterial etiology was shigellosis, which was also implicated in cases of reactive arthritis.[5,102,133] Bacterial

Table 2. Amount of literature coverage afforded to each etiology, displayed as absolute number and percentage of total ($n = 174$), ordered alphabetically.

Etiologic category	Absolute number	% of total
Behavioral/psychological	8	5%
Congenital/hereditary	6	3%
Degenerative	11	6%
Husbandry related	9	5%
Idiopathic	30	17%
Immune mediated	5	3%
Infectious	62	36%
Metabolic	6	3%
Miscellaneous	4	2%
Neoplastic	15	9%
Trauma–accidental death	7	4%
Vascular	11	6%

Table 3. Infectious disease, broken down by pathogenic agent, displayed as percentage of total ($n = 62$), ordered alphabetically.

Pathogenic agent	% of total
Bacterial	45%
Fungal	5%
Mixed	8%
Undetermined	6%
Viral	15%

infections were also commonly implicated in respiratory disorders, which were particularly frequently reported in orangutans.[17,73,123,137] *Balantidium coli* and *Balamuthia mandrillaris* were the most frequently cited parasitic infections. Viral diseases reported, amongst others, included cases of fatal viral myocarditis[61,88,93,136] and also herpesvirus infection.[66,108] Respiratory syncytial virus (RSV), often in association with concurrent *Streptococcus pneumoniae* infection, was reported to cause severe, often fatal, bronchopneumonia.[27,126,129] Fungal infections including coccidioidomycosis[49,52] and a suspected case of dermatophilosis[14] were also reported. Human-to-great ape zoonotic disease transmission reported in the literature included cases of respiratory disease due to RSV, *Streptococcus pneumoniae*, and whooping cough, and cases of coxsackie B3 virus, *Entamoeba histolytica*, and varicella virus infection.[30,46,79,93,126,129]

The majority of those articles classified as being on the topic of idiopathic disorders were reporting on the occurrence of cardiovascular disease. Reports identified suggest that disorders of the cardiovascular system are associated with high rates of mortality in at least three (chimpanzees, gorillas, and orangutans) of the four great ape taxa. However, there is very little evidence presented in the literature about the epidemiology, diagnosis, and treatment of great ape cardiovascular disease, suggesting that current understanding remains poor and that there is a critical need for further research in this area. Other reported idiopathic conditions were varied, and included epilepsy, appendicitis, hyperthyroidism, and diverticulitis.[33,62,80,91]

The female reproductive tract[30,54,116,124] and gastrointestinal system were the body systems most commonly reported to be affected by neoplastic disorders[26,111] (Table 4).

Body system: A total of 177 (97%) of the 183 papers could be classified according to a single body-system category. The body systems most commonly reported on were cardiovascular

Table 4. Neoplastic disorders reported in the literature with references (ref.), by taxa, listed alphabetically.

Taxa	Condition	Ref.
Chimpanzee	Gastrointestinal stromal tumor	110
	Gingival mass	109
	Hepatocellular carcinoma and myelolipoma	99
	Maxillary sarcoma	41
	Nevus lipomatosus cutaneus superficialis	67
	Renal carcinoma	44
	Uterine leiomyoma	116
Gorilla	Acute lymphocytic leukemia	7
	Choriocarcinoma	30
	Inoperable obstetric cancer	34
	Intracranial tumor gorilla (possible lymphoma)	82
	Leydigocytoma and a large cell lung carcinoma	96
	Metastatic pancreatic islet cell carcinoma	26
	Primary hyperparathyroidism (presumed adenoma)	33
	Prolactin secreting pituitary adenoma	23
	Squamous cell carcinoma of the skin	40
	Uterine adenocarcinoma and squamous cell carcinoma of vagina, cervix, and uterus	124
Orangutan	Malignant gastric rhabdoid tumor	111

(18%), generalized–multi-system (18%), gastrointestinal (12%), and respiratory (11%).

Cardiovascular: Idiopathic myocardial fibrosis (often referred to as fibrosing cardiomyopathy) was reported among gorillas, chimpanzees, and orangutans and was associated with sudden death, peri-anesthetic death, congestive heart failure, and cerebrovascular infarction.[26,70,85,90,113] Other cardiomyopathies reported include dilated cardiomyopathy[118,119] and arrhythmogenic right-ventricular cardiomyopathy.[127] Several reports of aortic aneurysm or dissection in male gorillas[3,64] and cardiac arrhythmias, particularly among male chimpanzees, also featured.[36,69,120] Hypertension was reported to affect gorillas and chimpanzees and has been shown to correlate with age and obesity and to increase mortality risk.[36,37,85] Other reports included congenital heart disease,[43,128] fatal myocarditis,[88,93,103,136] a case of coronary artery disease in a gorilla,[114] and three cases of cerebrovascular accident (stroke) in female chimpanzees.[60]

Generalized–multi-system: This category consisted largely of infectious disorders. Noninfectious conditions in this category included cases of idiopathic hypocalcaemia, Reyes-like syndrome, systemic anaphylaxis, protein deficiency, clinically significant chromosomal abnormalities, and intra-abdominal abscesses or cysts.[12,15,24,48,72,86,89,92] Obesity (with or without concurrent metabolic syndrome) was also categorized as a generalized condition.[74,94,122,131]

Gastrointestinal: Most reported gastrointestinal diseases were infection-associated. The most commonly reported presenting sign was diarrhea, which was occasionally hemorrhagic,[57,71,95,97] but lethargy, inappetence, anorexia, tenesmus, rectal prolapse, and abdominal pain also featured.[33,91] Three cases of neoplasia of the gastrointestinal system (Table 5) and one case of paralytic ileus in an orangutan, which occurred secondary to severe depression, were also reported.[121]

Respiratory: Air sacculitis was the most frequently discussed respiratory disorder, especially in orangutans.[17,73,105,127,138] RSV, with or without concurrent *Streptococcus pneumoniae* infection, was also identified as a common cause of fatal disease.[27,126,129] Other infectious agents reported included *Bordetella pertussis*,[66] *Streptococcus anginosus*,[55] *Mycobacterium kansasii*,[4] and *Mycobacterium tuberculosis*.[115,117] Noninfectious disorders included allergic respiratory disease and anesthetic complications.[35,63,65] Clinical signs included nasal discharge, coughing, tachypnea, dyspnea, and, less commonly, facial swelling, exophthalmos, and cyanosis.

Musculoskeletal: The majority of musculoskeletal disorders reported were degenerative in origin.[1,19,132,134] Rickets was reported in a gorilla, an orangutan, and a group of chimpanzees.[8,22,62]

Neurologic: Four great apes (one orangutan; three gorillas) displayed nonspecific clinical signs progressing to ataxia, disorientation, and death caused by the amoeba species *Balamuthia mandrillaris*.[18,78,104] Encephalopathy was also the cause of death in a case of septicemia due to *Aeromonas hydrophilia* infection in a gorilla.[47] Noninfectious

Table 5. Amount of literature coverage afforded to each body system, displayed as absolute number and percentage of total ($n = 177$), ordered alphabetically.

Body system category	Absolute number	% of total
Behavioral	10	6%
Cardiovascular	31	18%
Dental	3	2%
Endocrine	8	5%
Gastrointestinal	21	12%
Generalized–multi-system	31	18%
Hepatobiliary	1	<1%
Hematopoietic–lymphatic	1	<1%
Musculoskeletal	14	8%
Neurological	12	7%
Ophthalmic	6	3%
Renal	2	1%
Reproductive	9	5%
Respiratory	20	11%
Skin–integumentary	6	3%
Urinary (lower)	2	1%

neurologic conditions reported include inflammatory polyradiculoneuropathy, cerebral calcinosis, vascular mineralization, and acute transverse myelitis in chimpanzees;[2,28,87,139] intracranial mass, hydrocephalus, and age-related pallido-nigral degeneration in gorillas;[77,82] demyelinating polyneuropathy in an orangutan;[31] and epilepsy in bonobos.[42]

Behavioral: One observational study of zoo-housed chimpanzees concluded that abnormal behavior is endemic in the population.[11] The paper reported that all 40 animals displayed at least two abnormal behaviors, including coprophagy, stereotypic grooming, genital touching, rocking, regurgitation, hair plucking, and self-injury. Regurgitation–reingestion syndrome was also reported in gorillas,[51,75] chimpanzees,[125] and orangutans.[20] Self-injurious behavior was identified in a gorilla,[38] a chimpanzee,[13] and a bonobo.[101] Mood and anxiety disorders were also described in chimpanzees.[16,59]

Other body systems: Disorders of the integument, reproductive and genitourinary tracts, and endocrine, hepatobiliary, and hematopoietic-lymphatic systems as well as ophthalmic and dental conditions each constituted five percent or less of the articles identified

Whilst these figures relating to the frequency of reporting are informative, it must be remembered that amount of literature coverage a disorder receives might be influenced by a number of factors; for example, the veterinary knowledge and diagnostic capabilities available at the time, the level of allocated funding, the specific clinical and academic interests of the author, or simply the author's perceived importance of the condition. Perhaps almost ironically, an author's perception of a condition's importance can in turn be influenced by the amount of literature coverage it receives. Case reports are an invaluable resource but they give very little perspective on the significance of a condition at a population level. An appreciation of this latter point requires in depth epidemiologic study and the calculation of comparative prevalence of various diseases and disorders. This was the purpose of just six of the records identified by the literature search (Table 6).

Comparative morbidity and mortality studies

Six (3%) of the 189 papers identified consisted of reviews of the various causes of morbidity and mortality among captive chimpanzees, gorillas, and orangutans. The main findings of each are summarized in Table 6.

Three of the six prevalence reviews were single-center studies.[68,90,94] The three remaining studies reviewed morbidity and mortality across more than one institution.[50,81,130] This allowed for a larger number of animals to be studied, arguably deeming the findings more representative of the wider population. Five of the six were retrospective reviews,[50,81,90,130] involving the use of routine information that had been collected for another purpose. The researchers therefore had very little control over the accuracy and completeness of the dataset, which might negatively impact upon the reliability of the findings. In contrast, the screening of 16 geriatric female chimpanzees for the presence of chronic and age-related disease[94] was a prospective study and therefore had the advantage that data collection methods were specifically designed for a purpose. The results of this study might therefore be considered more reliable, although the potential for observer bias (bias introduced due to the investigator's prior knowledge of the hypothesis under investigation of the individual's exposure or disease status)[6] remains. All six studies reported the frequency of occurrence of various conditions but were only able to speculate about underlying causes and risk factors.

Inclusion criteria were not always stated, making it difficult to ascertain the degree of selection bias present. In two of the multi-center studies,[50,81] the investigators were reliant on various institutions submitting data to the study.

Table 6. Summary of key components of the six morbidity–mortality reviews identified by the literature search.

Author and year	Period of study	Study type	Study population	Main causes of morbidity–mortality
Meehan and Lowenstine 1994[81]	1980–1994	Retrospective, observational, longitudinal, multi-center (unknown no.); review of postmortem records	Gorillas (n = 74); all ages, genders; Species Survival Plan population	Infants: trauma (60%) Adults (<30 yr): gastrointestinal (36%), cardiovascular (32%) Elderly (>30 yr): cardiovascular (53%)
Varki et al. 2009[110]	1966–1991	Retrospective, observational, longitudinal, multi-center (2); review of postmortem records	Chimpanzees (n = 58); adult (>10 yr); Yerkes National Primate Research Center; Primate Foundation of Arizona	1966–1991: enterocolitis (16%), heart disease (11%), meningitis (11%), pneumonia (5%), renal disease (5%), trauma (3%), miscellaneous (50%)
	1992–2008			1992–2008: heart disease (36%), renal disease (16%), trauma (12%), miscellaneous (36%)
Nunamaker et al. 2012[84]	2009	Prospective, observational, cross-sectional single center; incidence of disease at routine health assessment	Chimpanzees (n = 16); aged >35 yr; females; Alamogordo Primate Facility	Cardiovascular disease (81%), metabolic syndrome (44%), renal disease (31%)
Munson and Montali 1990[90]	Unknown	Retrospective, observational, longitudinal, single center; review of pathology and medical records	Gorillas, orangutans and chimpanzees (n = unknown); National Zoological Park, Washington, D.C.	Orangutans: myocardial fibrosis, gastrointestinal disease, perinatal infections Gorillas: arthritis, gastrointestinal disease, infertility, Chimpanzee: myocardial fibrosis
Lammey et al. 2008[68]	2001–2006	Retrospective, observational, longitudinal, single center; review of postmortem records	Chimpanzees (n = 36); aged 10–40 yr; Alamogordo Primate Facility	Sudden cardiac death (36%), renal failure (25%), trauma and septicemia (each 23%), anesthetic complications and neoplasia (each 15%)
Hewitt 2005[50]	1896–2005	Retrospective, observational, longitudinal, multi-center (9); review of postmortem records	Gorillas (n = 109); all ages; United Kingdom and Ireland populations (various zoological collections)	Respiratory (27%), multi-system (26%), gastrointestinal (15%) (cardiovascular disease: <9%)

This self-selection process has the potential to introduce bias but in retrospective studies that involve voluntary participation, it is largely unavoidable.

In five of the six studies[50,68,81,90,110] the data collected consisted of medical records or pathology reports written by various clinicians and pathologists at the time of an animal's illness or

death. In one study,[50] reported morbidity and mortality events date as far back as 1896, when veterinary diagnostic capabilities were limited.

In view of the small sample sizes, the time span, and the single-center focus of the studies, they may not be representative on a wider population level, and they may not provide an accurate and up-to-date representation of the current situation.

Limitations of this systematic literature review

The validity of the conclusions depends heavily on the reliability of the initial literature search. Every effort was made to make the search as exhaustive as possible though the use of synonyms and Medical Subject Headings (MeSH) terms. The search strategy used within this review returned a total of 1,146 initial results, which were considered to constitute a representative proportion of the literature body as a whole. Less than one-third ($n = 313$) of the 1,146 results were duplicates, suggesting that there is a relatively low degree of overlap of coverage by the databases accessed. Some relevant papers were not identified by the literature search;[23,76,98] this might have been due to words (for example, "captive" or "zoo") not being included in the key words provided by authors, or at the point of database indexing. This highlights the importance of the application of accurate and representative key words by journal authors and editors in order to facilitate such search and review processes. These omissions might, however, simply reflect the inherent limitations of any literature search, and the difficulties associated with striking the balance between maximizing sensitivity whilst retaining specificity.

For case report–case series or single diseases, category definitions were described and closely adhered to but some degree of subjectivity and ambiguity was still encountered. An example of this is regurgitation–reingestion, categorized in this review as a behavioral disorder. However, since diet and environmental conditions have been suggested as underlying causes, it might have been categorized as husbandry-related, illustrating how easily categorization can influence results.

Differences in the approach to categorization might also have been responsible for the variation in findings between the two gorilla comparative mortality studies identified by the search: Meehan and Lowenstine[81] found that cardiovascular disease was responsible for 32% of adult and 41% of aged gorilla deaths. However, Hewitt[50] identified it as the cause of death in less than 9% of the animals. If this discrepancy were genuine, it might suggest that there are differences in the cardiac risk factors (such as genetics, diet, husbandry) to which the North American and United Kingdom–Irish populations are exposed. Upon closer examination, however, a number of animals included in the latter study were diagnosed as having cardiovascular and another concurrent disease on postmortem examination. These deaths were categorized as being multi-system in origin, which might have led to underrepresentation of the importance of cardiovascular disease as a cause of mortality within this population.

Categorization according to etiology does not account for disorders or events that occur due to a more complicated, multi-factorial pathophysiology. Examples of such are anesthesia-related complications and infection-associated disorders. In the latter case, the pathogen may not be the primary or sole cause; the infection might be a complicating factor in another disease process or may be occurring secondarily to underlying reasons such as immunosuppression, poor husbandry, or environmental conditions. In human medicine, many of the issues highlighted here are overcome by the implementation of the International Classification of Diseases system.[135] This might in fact be a more appropriate categorization model for morbidity and mortality reviews in veterinary medicine to utilize in the future.

CONCLUSIONS

This is the first review of the great ape literature published since 1990 and the first-ever systematic literature review of great ape morbidity and mortality. The review highlights cardiovascular disease, multi-system disorders, and infectious diseases as areas of current topical interest within the current great ape literature. It identifies cardiovascular and other chronic or age-related diseases to be of particular importance, especially in light of the increasing longevity of great apes housed in modern zoos.

The review concludes, however, that there is a critical need for a robust, widespread and more up-to-date review of mortality among captive great apes. The aims of such a review would be to identify the main causes of morbidity and mortality for each age group and species. Any retrospective review performed should focus upon a relatively recent time period in order to produce findings that provide an accurate and reliable representation of the current situation. Prospectively, data collection methods should provide the opportunity for more an in-depth

study of subpopulations, such as specific age groups (e.g., infant or geriatric animals), between which patterns of disease and mortality are likely to differ. In particular, in-depth study of the risk factors associated with the development of diseases of significance would be especially interesting, as would comparison of mortality between captive and wild great apes. The findings of such a study would be informative, not only for the practicing clinician, but also in providing a focus for further investigation and research to help improve captive great ape health and welfare. Working closely with the Great Ape TAG, studbook coordinators, and vet advisors would facilitate data collection and maximize the potential benefits of such a study.

Acknowledgments: Thanks go to supervisors and colleagues at the named institutions for their ongoing support and assistance.

LITERATURE CITED

1. Aldridge VJ. A search for probable cause: self-wounding in a zoo chimpanzee (*Pan troglodytes*). Am J Primatol. 2005;66(Suppl. 1):82 (Meeting Abstr.).

2. Alford PL, Satterfield WC. Paralytic illness resembling inflammatory polyradiculoneuropathy in a chimpanzee. J Am Vet Med Assoc. 1995;207(1):83–85.

3. Allchurch AF. Sudden death and cardiovascular disease in the lowland gorilla. Dodo. 1993;29:172–178.

4. Alvarado T. Atypical mycobacterial pneumonia in a western lowland gorilla caused by *Mycobacterium kansasii*. In: AAZPA Regional Conf Proc; 1992. p. 629–632.

5. Banish LD, Bush M, Montali RJ, Sac D. Shigellosis in a zoological collection of primates. J Zoo Wildl Med. 1990;21:302–309.

6. Barratt H, Kirwan M. Biases: bias in epidemiological studies [Internet]. Health Knowledge. 2009 [cited 2015 Jul 15]. Available from: http://www.healthknowledge.org.uk/public-health-textbook/research-methods/1a-epidemiology/biases

7. Barrie MT, Backeus KA, Grunow J, Nitschke R. Acute lymphocytic leukemia in a six-month-old western lowland gorilla (*Gorilla gorilla gorilla*). J Zoo Wildl Med [Internet]. 1999;30:268–272. Available from: <Go to ISI>://000081607900011

8. Bellisari A, Duren DL, Sherwood RJ, Barrie M. A captive infant female gorilla with vitamin D and calcium deficiency: preliminary description. Am J Phys Anthropol. 2004;S38:61.

9. Benirschke K, Adams FD. Gorilla diseases and causes of death. J Reprod Fertil Suppl. 1980;Suppl 28:139–148.

10. Beutin L, Knollmann-Schanbacher G, Rietschel W, Seeger H. Animal reservoirs of *Escherichia coli* O157:[H7]. Vet Rec. 1996;139(3):70–71.

11. Birkett LP, Newton-Fisher NE. How abnormal is the behaviour of captive, zoo-living chimpanzees? PLoS One. 2011;6(6):e20101.

12. Blampied N le Q, De K, Fullerton D, Coleman P, Purcell-Jones G, Carroll B. Surgical removal of an abdominal cyst from a Bornean orang-utan *Pongo pygmaeus pygmaeus*. Dodo. 1992;28:165–169.

13. Bourgeois SR, Vazquez M, Brasky K. Combination therapy reduces self-injurious behavior in a chimpanzee (*Pan troglodytes troglodytes*): a case report. J Appl Anim Welf Sci. 2007;10(2):123–140.

14. Brack M, Hochleithner C, Hochleithner M, Zenker W. Suspected dermatophilosis in an adult orangutan (*Pongo pygmaeus pygmaeus*). J Zoo Wildl Med. 1997;28:336–341.

15. Bradford CM, Tupa L, Wiese D, Hurley TJ, Zimmerman R. Unusual Turner syndrome mosaic with a triple x cell line (47,X/49,XXX) in a western lowland gorilla (*Gorilla gorilla gorilla*). J Zoo Wildl Med. 2013;44:1055–1058.

16. Bradshaw GA, Capaldo T, Lindner L, Grow G. Building an inner sanctuary: complex PTSD in chimpanzees. J Trauma Dissociation. 2008;9:9–34.

17. Cambre RC, Edwards JE, Wilson HL, Todd JK, Strain JD, Hendee RW, Jaskunas JM, Knox RF, Chang JHT. Maxillary and ethmoid sinusitis with orbital and intracranial extension in an infant orangutan (*Pongo pygmaeus*). J Zoo Wildl Med. 1995;26:144–151.

18. Canfield PJ, Vogelnest L, Cunningham MI, Visvesvara GS. Amoebic meningoencephalitis caused by *Balamuthia mandrillaris* in an orangutan. Aust Vet J. 1997;75(2):97–100.

19. Carter ML. Skeletal and dental pathology of wild and captive chimpanzees Clinical and evolutionary implications. Am J Phys Anthropol. 1992;90(S16):67–68.

20. Cassella CM, Mills A, Lukas KE. Prevalence of regurgitation and reingestion in orangutans housed in North American zoos and an examination of factors influencing its occurrence in a single group of Bornean orangutans. Zoo Biol. 2012;31(5):609–620.

21. Centre for Reviews and Dissemination. Systematic reviews: CRD's guidance for undertaking reviews in health care. York (United Kingdom) CRD, University of York; 2009.Chapter 1, p. 6–14.

22. Chai N, Bomsel MC, Berthier JL. A case of osteodystrophy in an orang-utan. In: Erkrankungen der Zootiere: Verhandlungsbericht des 41 Internationalen Symposiums uber die Erkrankungen der Zoo- und Wildtiere; 2003. p. 81–86.

23. Chai N, Hazan T, Wedlarski R, Rigoulet J. Treatment of a retroperitoneal abscess by omentalization in an orangutan (*Pongo pygmaeus pygmaeus*). J Zoo Wildl Med. 2009;40:350–353.

24. Chatfield J, Stones G, Jalil T. Severe idiopathic hypocalcemia in a juvenile western lowland gorilla, *Gorilla gorilla gorilla*. J Zoo Wildl Med. 2012;43:171–173.

25. Chatfield J, Zhang L, Ramey J, Bowsher T, Loskutoff N, O'Neill K. Resolution of a hyperprolactinemia in a western lowland gorilla (*Gorilla gorilla gorilla*). J Zoo Wildl Med. 2006;37:565–566.

26. Chiu E, Bodley K. A psychogeriatrician's home visit to the zoo: a case report. Int Psychogeriatrics. 2010;22:671–673.

27. Clarke CJ, Watt NJ, Meredith A, McIntyre N, Burns SM. Respiratory syncytial virus-associated bronchopneumonia in a young chimpanzee. J Comp Pathol. 1994;110:207–212.

28. Connor-Stroud FR, Hopkins WD, Preuss TM, Johnson Z, Zhang X, Sharma P. Extensive vascular mineralization in the brain of a chimpanzee (*Pan troglodytes*). Comp Med [Internet]. 2014 [cited 2014 Aug 25]; 64(3):224–229. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24956215

29. Convention on International Trade in Endangered Species (CITES). Convention on International Trade in Endangered Species of Wild Fauna and Flora: Appendices I, II and III [Internet]. (cited 2016 July 18) 2015. Available from: http://www.cites.org/sites/default/files/eng/app/2015/E-Appendices-2015-02-05.pdf

30. Cook RA, Calle PP, Mangold B, McNamara T, Raphael B, Stetter M, Goldstein L, Haramati N, Barakat R, Jones W, Al. E. Choriocarcinoma in a young adult gorilla (*Gorilla g. gorilla*): diagnosis, treatment and outcome. In: American Association of Zoo Veterinarians Annual Conference, American Association of Zoo Veterinarians; 2010. p. 229–230.

31. Cossaboon C, Antilla A, Geurts T, Pietsch R. Allie's story: the road to recovery. A seven year history of a Sumatran orangutan (*Pongo abelii*) with a neurological disease. Anim Keepers' Forum. 2008;35:273–280.

32. Cousins D. Mortality factors in captive gorillas (*Gorilla gorilla*). Int Zoo News. 1983;30:5–17.

33. D'Agostino J, Isaza R, Fingland R, Hoskinson J, Ragsdale J. Acute appendicitis in a chimpanzee (*Pan troglodytes*). J Med Primatol. 2007;36(3):119–123.

34. Doane CJ, Lee DR, Sleeper MM. Electrocardiogram abnormalities in captive chimpanzees (*Pan troglodytes*). Comp Med. 2006;56:512–518.

35. Dumonceaux GA, Lamberski N, Clutter D, Nagy SMJ, Burek K, Phillips LG. Treatment of bilateral nasal polyposis and chronic refractory inhalant allergic rhinitis in a chimpanzee (*Pan troglodytes*). J Zoo Wildl Med. 1997;28:215–219.

36. Ely JJ, Zavaskis T, Lammey ML. Hypertension increases with aging and obesity in chimpanzees (*Pan troglodytes*). Zoo Biol. 2013;32:79–87.

37. Ely JJ, Zavaskis T, Lammey ML, Rick Lee D. Blood pressure reference intervals for healthy adult chimpanzees (*Pan troglodytes*). J Med Primatol. 2011; 40:171–180.

38. Espinosa-Avilés D, Elizondo G, Morales-Martínez, M Rodríguez-Herrejón F, Varela P. Treatment of acute self-aggressive behaviour in a captive gorilla (*Gorilla gorilla gorilla*). Vet Rec. 2004;154:401–402.

39. Ferdowsian HR, Durham DL, Kimwele C, Kranendonk G, Otali E, Akugizibwe T, Mulcahy JB, Ajarova L, Johnson CM. Signs of mood and anxiety disorders in chimpanzees. PLoS One. 2011;6.

40. Fernandez Bellon H, Vidal M, Borau J, Fernandez A, Moran J. Squamous cell carcinoma of the skin in an albino gorilla (*Gorilla gorilla gorilla*). In: Proceedings of the Institute for Zoo and Wildlife Research No 5; 2003. p. 175–177.

41. Fujisawa M, Udono T, Nogami E, Hirosawa M, Morimura N, Saito A, Seres M, Teramoto M, Nagano K, Mori Y, Uesaka H, Nasu K, Tomonaga M, Idani G, Hirata S, Tsuruyama T, Matsubayashi K. A case of maxillary sarcoma in a chimpanzee (*Pan troglodytes*). J Med Primatol. 2014;43(2):111–114.

42. Gerlach T, Clyde VL, Morris GL, Bell B, Wallace RS. Alternative therapeutic options for medical management of epilepsy in apes. J Zoo Wildl Med. 2011;42:291–294.

43. Greenberg M, Jansse D, Jamieson S, Rothman A, Frankville D, Cooper S, Kriett J, Adsit P, Shima A, Morris P, Sutherland-Smith M. Surgical repair of an atrial septal defect in a juvenile Sumatran orangutan (*Pongo pygmaeus sumatruensis*). J Zoo Wildl Med. 1999; 30:256–261.

44. Greenwood AG, Lowe JW, Gaunt L. Renal carcinoma in a chimpanzee (*Pan troglodytes*). Vet Rec. 1995;137:380–381.

45. Grindlay DJC, Brennan ML, Dean RS. Searching the veterinary literature: a comparison of the coverage of veterinary journals by nine bibliographic databases. J Vet Med Ed. 2012; p. 1–9.

46. Gustavsson OEA, Röken BO, Serrander R. An epizootic of whooping cough among chimpanzees in a zoo. Folia Primatol. 1990;55(1):45–50.

47. Harrison TM, Shellabarger WC, Reichard TA. Aeromonas hydrophila septicemia and resulting encephalopathy in a captive juvenile western lowland gorilla (*Gorilla gorilla gorilla*). In: Baer CK; Willette MM (eds.). Proceedings American Association of Zoo Veterinarians, American Association of Wildlife Veterinarians, Association of Reptilian and Amphibian Veterinarians, National Association of Zoo and Wildlife Veterinarians Joint Conference. American Association of Zoo Veterinarians; 2001. p. 366–370.

48. Hayman DT, King T, Cameron K. Successful treatment of acute systemic anaphylaxis in a western lowland gorilla (*Gorilla gorilla gorilla*). J Zoo Wildl Med. 2010;41:522–525.

49. Herrin KV, Miranda A, Loebenberg D. Posaconazole therapy for systemic coccidioidomycosis in a chimpanzee (*Pan troglodytes*): a case report. Mycoses. 2005;48(6):447–452.

50. Hewitt S. Preliminary findings of the prevalence of diseases in captive western lowland gorillas (*Gorilla gorilla gorilla*) in the United Kingdom and Ireland

(1896–2005). In: Proceedings of the 7th Annual Symposium on Zoo Research; 2005. p. 222–231.

51. Hill SP. Do gorillas regurgitate potentially-injurious stomach acid during regurgitation and re-ingestion? Anim Welf. 2009;18:123–127.

52. Hoffman K, Videan EN, Fritz J, Murphy J. Diagnosis and treatment of ocular coccidioidomycosis in a female captive chimpanzee (*Pan troglodytes*): a case study. Ann N Y Acad Sci [Internet]. 2007 [cited 2014 Aug 23];1111:404–410. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17303834

53. Hooper J. Primary hyperparathyroidism in a western lowland gorilla. In: Roberts V (ed.). In: British Veterinary Zoological Society Proceedings of the Spring Meeting: Mammalian Medicine and Surgery; 2011. p. 23.

54. Hunter R, Agnew A. A differential diagnosis of diffuse idiopathic skeletal hyperostosis (DISH) in a *Gorilla gorilla gorilla* specimen. Am J Anthropol 80th Annu Meet Am. 2011;144(S52):172.

55. Ihms EA, Daniels JB, Koivisto CS, Barrie MT, Russell DS. Fatal *Streptococcus anginosus*–associated pneumonia in a captive Sumatran orangutan (*Pongo abelii*). Zoo Biol. 2006;25(2):101–115.

56. International Union for Conservation of Nature [Internet]. The IUCN Red List of Threatened Species. Version 2013. c2013 [cited 2013 Nov 11]. Available from: <http://www.iucnredlist.org>

57. Isidoro M, Martinez J, Ramis A. Haemorrhagic-necrotizing colitis associated with *Campylobacter jejuni* and *Balantidium coli* in a zoo gorilla. J Comp Pathol. 2013;148(1):87.

58. Janssen DL. Diseases of great apes. In: Fowler ME (ed.). Zoo and wild animal medicine: current therapy 3. Philadelphia (PA): W.B. Saunders; 1993. p. 334–338.

59. Janssen DL, Bush RM. Review of the medical literature of great apes in the 1980s. Zoo Biol. 1990;9:123–134.

60. Jean SM, Preuss TM, Sharma P, Anderson DC, Provenzale JM, Strobert E, Ross SR, Stroud FC. Cerebrovascular accident (stroke) in captive, group-housed, female chimpanzees. Comp Med. 2012;62(4):322–329.

61. Jones P, Cordonnier N, Mahamba C, Burt FJ, Rakotovao F, Swanepoel R, André C, Dauger S, Bakkali Kassimi L. Encephalomyocarditis virus mortality in semi-wild bonobos (*Pan panicus*). J Med Primatol. 2011;40:157–163.

62. Junge RE, Gannon FH, Porton I, McAlister WH, Whyte MP. Management and prevention of vitamin D deficiency rickets in captive-born juvenile chimpanzees (*Pan troglodytes*). J Zoo Wildl Med. 2000;31:361–369.

63. Karesh WB, Lidell RM, Sirotta P. Clinical challenge case 1: aspiration pneumonia and abscess in a gorilla. Primates. 2012;53(3):221–226.

64. Kenny DE, Cambre RC, Alvarado TP, Prowten AW, Allchurch AF, Marks SK, Zuba JR. Aortic dissection—an important cardiovascular-disease in captive gorillas (*Gorilla gorilla gorilla*). J Zoo Wildl Med. 1994;25:561–568.

65. Kenny DE, Knightly F, Haas B, Hergott L, Kutinsky I, Eller JL. Negative-pressure pulmonary edema complicated by acute respiratory distress syndrome in an orangutan (*Pongo pygmaeus abelii*). J Zoo Wildl Med. 2003;34:394–399.

66. Kik MJ, Bos JH, Groen J, Dorrestein GM. *Herpes simplex* infection in a juvenile orangutan (*Pongo pygmaeus pygmaeus*). J Zoo Wildl Med. 2005;36:131–134.

67. Klopfleisch R, Langner C, Von Felbert I, Rudnick JC, Teifke JP. Nevus lipomatosus cutaneus superficialis (Hoffmann-Zurhelle) in a chimpanzee (*Pan troglodytes*). J Med Primatol. 2007;36:57–60.

68. Lammey ML, Baskin GB, Gigliotti AP, Lee DR, Ely JJ, Sleeper MM. Interstitial myocardial fibrosis in a captive chimpanzee (*Pan troglodytes*) population. Comp Med. 2008;58:389–394.

69. Lammey ML, Jackson R, Ely JJ, Lee DR, Sleeper MM. Use of an implantable loop recorder in the investigation of arrhythmias in adult captive chimpanzees (*Pan troglodytes*). Comp Med. 2011;61:71–75.

70. Lammey ML, Lee DR, Ely JJ, Sleeper MM. Sudden cardiac death in 13 captive chimpanzees (*Pan troglodytes*). J Med Primatol [Internet]. 2008 [cited 2013 Nov 20];37 Suppl 1(13):39–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18269527

71. Lankester F, Mätz-Rensing K, Kiyang J, Jensen SA, Weiss S, Leendertz FH. Fatal ulcerative colitis in a western lowland gorilla (*Gorilla gorilla gorilla*). J Med Primatol. 2008;37(6):287–302.

72. Lear TL, Houck ML, Zhang YW, Debnar LA, Sutherland-Smith MR, Young L, Jones KL, Benirschke K. Trisomy 17 in a bonobo (*Pan paniscus*) and deletion of 3q in a lowland gorilla *(Gorilla gorilla gorilla)*: comparison with human trisomy 18 and human deletion 4q syndrome. Cytogenet Cell Genet. 2001;95(3–4):228–233.

73. Lewis I, Lung NP, Ward A. Management of a chronically ill orangutan: communication and cooperation are the keys. In: Brookfield Zoo (ed.). The apes: challenges for the 21st century. Conference proceedings Chicago Zoological Society; 2001. p. 186–189.

74. Lintzenich B, Ward AM. Weight management strategies in apes. In: Brookfield Zoo (ed.). The apes: challenges for the 21st century. Conference proceedings Chicago Zoological Society; 2001. p. 175–176.

75. Lukas KE, Hamor G, Bloomsmith MA, Horton CL, Maple TL. Removing milk from captive gorilla diets: the impact on regurgitation and reingestion (R/R) and other behaviors. Zoo Biol. 1999;18(6):515–528.

76. Lung N, Miller J, Ferrell S, Marlar A, Turner L. Surgical and medical management of necrotizing fasciitis in the throat sac region of an adult male Sumatran orangutan (*Pongo pygmaeus abelii*). In: Proc Am Assoc Zoo Veterinarians; 2004. p. 195.

77. Márquez M, Serafin A, Fernández-Bellon H, Serrat S, Ferrer-Admetlla A, Bertranpetit J, Ferrer I, Pumarola M. Neuropathologic findings in an aged albino gorilla. Vet Pathol [Internet]. 2008 [cited 2014 Jul 18];45(4):531–537. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18587101

78. Mätz-Rensing K, Kunze M, Zöller M, Roos C, Kiderlen AF, Ludwig C, Kaup F-J. Fatal *Balamuthia mandrillaris* infection in a gorilla—first case of balamuthiasis in Germany. J Med Primatol [Internet]. 2011 [cited 2014 Jul 18];40(6):437–440. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21496054

79. Mbaya AW, Nwosu CO. Outbreak of clinical amoebosis among captive chimpanzees (*Pan troglodytes*) at the Sanda Kyarima park, Maiduguri, Nigeria. Niger Vet J. 2005;27(1):68–73.

80. McLachlan SM, Alpi K, Rapoport B. Review and hypothesis: does Graves' disease develop in non-human great apes? Thyroid. 2011;21:1359–1366.

81. Meehan TP, Lowenstine LJ. Causes of mortality in captive lowland gorillas: a survey of the SSP population. In: Proc Am Assoc Zoo Veterinarians; 1994. p. 216–218.

82. Meehan TP, Zdziarski JM, Briggs MB, Anderson DB, Zelby AS, Grigg-Damberger, M Thomas C, Murnane RD, Walsh TM. Surgical removal of an intracranial tumor in a western lowland gporilla. In: Junge RE (ed.). Proceedings of a Joint Conference American Association of Zoo Veterinarians, Wildlife Disease Association, and American Association of Wildlife Veterinarians; 1995. p. 255–256.

83. Mesle F. Classifying causes of death according to an aetiological axis. Popul Stud (NY). 1999;53(1):97–105.

84. Microsoft. Microsoft Excel. Microsoft Office. Version 14.0.7147.5001 [Computer software]. Microsoft: Redmond (WA); 2010.

85. Miller CL, Schwartz AM, Barnhart JS, Bell MD. Chronic hypertension with subsequent congestive heart failure in a western lowland gorilla (*Gorilla gorilla gorilla*). J Zoo Wildl Med. 1999;30:262–267.

86. Minter LJ, Cullen JM, Loomis MR. Reye's or Reye's-like syndrome in western lowland gorilla (*Gorilla gorilla gorilla*). J Med Primatol. 2012;41:329–331.

87. Miyabe-Nishiwaki T, Kaneko A, Nishiwaki K, Watanabe A, Watanabe S, Maeda N, Kumazaki K, Morimoto M, Hirokawa R, Suzuki J, Ito Y, Hayashi M, Tanaka M, Tomonaga M, Matsuzawa T. Tetraparesis resembling acute transverse myelitis in a captive chimpanzee (*Pan troglodytes*): long-term care and recovery. J Med Primatol [Internet]. 2010 [cited 2014 Jul 18];39(5):336–346. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20444005

88. Miyagi J, Tsuhako K, Kinjo T, Iwamasa T, Kamada Y, Kinju T, Koyanagi Y. Coxsackievirus B4 myocarditis in an orangutan. Vet Pathol [Internet]. 1999 [cited 2013 Nov 26];36(5):452–456. Available from: http://vet.sagepub.com/lookup/doi/10.1354/vp.36-5-452

89. Mundy NI, Ancrenaz M, Wickings EJ, Lunn PG. Protein deficiency in a colony of western lowland gorillas (*Gorilla g. gorilla*). J Zoo Wildl Med. 1998;29:261–268.

90. Munson L, Montali RJ. Pathology and diseases of great apes at the National Zoological Park. Zoo Biol. 1990;9:99–105.

91. Murray S, Zdziarski JM, Bush M, Citino SB, Schulman FY, Montali R. Diverticulitis with rupture and fatal peritonitis in a Sumatran orangutan (*Pongo pygmaeus*). Comp Med. 2000;50(4):452–454.

92. Mylniczenko ND. A preliminary report on intra-abdominal abscesses in captive western lowland gorillas (*Gorilla gorilla gorilla*). In: Baer CK (ed.). American Association of Zoo Veterinarians Annual Conference; 2003. p. 62–66.

93. Nielsen SCA, Mourier T, Baandrup U, Søland TM, Bertelsen MF, Gilbert MTP, Nielsen LP. Probable transmission of coxsackie B3 virus from human to chimpanzee, Denmark. Emerg Inf Dis. 2012;18:1163–1165.

94. Nunamaker EA, Lee DR, Lammey ML. Chronic diseases in captive geriatric female chimpanzees (*Pan troglodytes*). Comp Med. 2012;62:131–136.

95. Paixao TA, Malta MCC, Soave SA, Tinoco HP, Costa MELT, Pessanha AT, Silva ROS, Coura FM, Costa LF, Turchetti AP, Lobato FCF, Melo MM, Santos, L. Renato, Heinemann MB. Hemorrhagic colitis associated with *Salmonella enterica* serotype Infantis infection in a captive western lowland gorilla (*Gorilla gorilla gorilla*) in Brazil. J Med Primatol. 2014;43(2):118–121.

96. Paixão TA, Tinoco HP, Campos M De, Elvira M, Teixeira L, Soave SA, Pessanha AT, Patrícia A, Silva C, Santos RL. Pathological findings in a captive senile western lowland gorilla (*Gorilla gorilla gorilla*) with chronic renal failure and septic polyarthritis. Brazilian J Vet Path; 2014;7:29–34.

97. Pazzaglia G, Widjaja S, Soebekti D, Tjaniadi P, Simanjuntak L, Lesmana M, Jennings G. Persistent, recurring diarrhea in a colony of orangutans (*Pongo pygmaeus*) caused by multiple strains of *Campylobacter* spp. Acta Trop. 1994;57(1):1–10.

98. Pollock PJ, Doyle R, Tobin E, Davison K BJ. Repeat laparotomy for the treatment of septic peritonitis in a Bornean orangutan (*Pongo pygmaeus pygmaeus*). J Zoo Wildl Med. 2008;39:476–479.

99. Porter B, Goens S, Brasky K, Hubbard G. A case report of hepatocellular carcinoma and focal nodular hyperplasia with a myelolipoma in two chimpanzees and a review of spontaneous hepatobiliary tumors in non-human primates. J Med Primatol. 2004;33(1):38–47.

100. PRISMA Group, Moher D, Liberati A, Tetzlaff J, Altman D. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement [Internet, cited 2015 September 15]. c2009. Available from: http://www.prisma-statement.org

101. Prosen H, Bell B. A psychiatrist consulting at the zoo (the therapy of Brian the bonobo). Brookfield Zoo (ed.). The apes: challenges for the 21st century Conference proceedings Chicago Zoological Society; 2001. p. 161–164.

102. Raphael BL, Calle PP, Haramati N, Watkins DI, Stetter MD, Cook RA. Reactive arthritis subsequent to *Shigella flexneri* enteritis in two juvenile lowland gorillas (*Gorilla gorilla gorilla*). J Zoo Wildl Med. 1995;26:132–138.

103. Reddacliff LA, Kirkland PD, Hartley WJ, Reece RL. Encephalomyocarditis virus infections in an Australian zoo. J Zoo Wildl Med. 1997;28:153–157.

104. Rideout BA, Gardiner CH, Stalis IH, Zuba JR, Hadfield T, Visvesvara GS. Fatal infections with *Balamuthia mandrillaris* (a free-living amoeba) in gorillas and other Old World primates. Vet Pathol. 1997;34: 15–22.

105. Rietschel W. Air sacculitis in bonobo (*Pan paniscus*) and chimpanzee (*Pan troglodytes*)—anatomy, pathology and therapy. In: Proc International Conference on Diseases of Zoo and Wild Animals; 2012. p. 112–115.

106. Rizzo F, Tappin SW, Tasker S. Thrombocytosis in cats: a retrospective study of 51 cases (2000–2005). J Feline Med Surg. 2007;9:319–325.

107. Robinson N, Dean RS, Cobb M, Brennan M. Investigating common clinical presentations in first opinion small animal consultations using direct observation. Vet Rec. 2015;176:463.

108. Sakulwira K, Theamboonlers A, Oraveerakul K, Chaiyabutr N, Bhattarakosol P, Poovorawan Y. Orangutan herpesvirus. J Med Primatol. 2004;33:25–29.

109. Sasseville VG, Mankowski JL, Baldessari A, Harbison C, Laing S, Kaliyaperumal S, Mätz-Rensing K, Miller AD, Schmidt LD, Kaplan-Kees J, Dick EJ, Reader JR, Liu D, Crawford LK, Lane JH, Corner SM, Pardo ID, Evans MG, Murnane R, Terio KA. Meeting report: emerging respiratory viral infections and nonhuman primate case reports. Vet Pathol [Internet]. 2013 [cited 2014 Aug 23];50(6):1145–1153. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23839235

110. Saturday GA, Lasota J, Frost D, Brasky KB, Hubbard G, Miettinen M. KIT-positive gastrointestinal stromal tumor in a 22-year-old male chimpanzee (*Pan troglodites*). Vet Pathol. 2005:362–365.

111. Schauer G, Moll R, Walter JH, Rumpelt HJ, Göltenboth R. Malignant rhabdoid tumor in the gastric wall of an aged orangutan (*Pongo pygmaeus*). Vet Pathol. 1994;31:510–517.

112. Schmidt RE. Systemic pathology of chimpanzees. J Med Primatol. 1975;7:274–318.

113. Schulman FY, Farb A, Virmani R, Montali RJ. Fibrosing cardiomyopathy in captive western lowland gorillas (*Gorilla gorilla gorilla*) in the United States—a retrospective study. J Zoo Wildl Med. 1995;26:43–51.

114. Scott NA, Mcmanamon R, Strobert E, Cipolla GD, Tarazona N, Swenson RB. In-vivo diagnosis of coronary-artery disease in a western lowland gorilla (*Gorilla gorilla gorilla*). J Zoo Wildl Med. 1995;26:139–143.

115. Shin NS, Kwon SW, Han DH, Bai GH, Yoon J, Cheon DS, Son YS, Ahn K, Chae C, Lee YS. Mycobacterium tuberculosis infection in an orangutan (*Pongo pygmaeus*). J Vet Med Sci. 1995;57:951–953.

116. Silva AE, Serakides NM, Ocarino GD, E.FC, Nascimento MA, Coradini R. Uterine leiomyoma in chimpanzee (*Pan troglodytes*). Arq Bras Med Vet e Zootec. 2006;58(1):129–132.

117. Singh KP, Singh R, Singh GR. Chronic pathological conditions encountered in captive wild mammals. Indian J Anim Sci. 1994;64:1346–1347.

118. Slaffer SN, Allchurch AF. Diagnosis and treatment of dilated (congestive) cardio-myopathy in a Sumatran orang-utan *Pongo pygmaeus abelii*. Dodo. 1995;31:147–152.

119. Sleeper MM, Doane CJ, Langner PH, Curtis S, Avila K, Lee DR. Successful treatment of idiopathic dilated cardiomyopathy in an adult chimpanzee (*Pan troglodytes*). Comp Med. 2005;55:80–84.

120. Sleeper MM, Drobatz K, Lee DR, Lammey ML. Echocardiographic parameters of clinically normal adult captive chimpanzees (*Pan troglodytes*). J Am Vet Med Assoc. 2014;244:956–960.

121. Sos E, Molnar V, Liptovszky M, Vrabely T, Rigo P. Veterinary aspects of a Sumatran orang-utan (*Pongo pygmaeus abelii*) introduction in a zoo situation. Erkrankungen der Zootiere. 2007;43:71–73.

122. Steinetz BG, Randolph C, Cohn D, Mahoney CJ. Lipoprotein profiles and glucose tolerance in lean and obese chimpanzees. J Med Primatol. 1996;25:17–25.

123. Steinmetz HW, Briner HR, Vogt R, Kalchofner K, Hilbe M, Scharf G, Eulenberger U, Hatt JM. Functional endoscopic sinus surgery in a Sumatran orang-utan (*Pongo pygmaeus abeli*). In: Wibbelt G, Bergholz N, Seet S, Hofer H (eds.). Proceedings of the Institute for Zoo and Wildlife Research, Berlin (7); 2007. p. 74–75.

124. Stringer EM, De Voe RS, Valea F, Toma S, Mulvaney G, Pruitt A, Troan B, Loomis MR. Medical and surgical management of reproductive neoplasia in two western lowland gorillas (*Gorilla gorilla gorilla*). J Med Primatol. 2010;39:328–335.

125. Struck K, Videan EN, Fritz J, Murphy J. Attempting to reduce regurgitation and reingestion in a captive chimpanzee through increased feeding opportunities: a case study. Lab Anim (NY). 2007;36(1):35–38.

126. Szentiks CA, Köndgen S, Silinski S, Speck S, Leendertz FH. Lethal pneumonia in a captive juvenile chimpanzee (*Pan troglodytes*) due to human-transmitted human respiratory syncytial virus (HRSV) and infection with *Streptococcus pneumoniae*. J Med Primatol. 2009;38(4):236–240.

127. Tong LJ, Flach EJ, Sheppard MN, Pocknell A, Banerjee AA, Boswood A, Bouts T, Routh A, Feltrer Y. Fatal arrhythmogenic right ventricular cardiomyopathy in 2 related subadult chimpanzees (*Pan troglodytes*). Vet

Pathol [Internet]. 2013 [cited 2013 Nov 20];51(4):858–867. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23988399

128. Trupkiewicz J, McNamara T, Weidenheim K, Cook R, Grenell S, Factor S. Cerebral infarction associated with coarctation of the aorta in a lowland gorilla (*Gorilla gorilla gorilla*). J Zoo Wildl Med. 1995; 26:123–131.

129. Unwin S, Chatterton J, Chantrey J. Management of severe respiratory tract disease caused by human respiratory syncytial virus and *Streptococcus pneumoniae* in captive chimpanzees (*Pan troglodytes*). J Zoo Wildl Med. 2013;44:105–115.

130. Varki N, Anderson D, Herndon JG, Pham T, Gregg CJ, Cheriyan M, Murphy J, Strobert E, Fritz J, Else JG, Varki A. Heart disease is common in humans and chimpanzees, but is caused by different pathological processes. Evol Appl [Internet]. 2009 [cited 2013 Nov 12];2(1):101–112. Available from: http://doi.wiley.com/10.1111/j.1752-4571.2008.00064.x

131. Videan EN, Fritz J, Murphy J. Development of guidelines for assessing obesity in captive chimpanzees (*Pan troglodytes*). Zoo Biol. 2007;26:93–104.

132. Videan EN, Satterfield WC, Buchl S, Lammey ML. Diagnosis and prevalence of uterine leiomyomata in female chimpanzees (*Pan troglodytes*). Am J Primatol. 2011;73:665–670.

133. Vielgrader H, Kubber-Heiss A, Voracek T, Alton K, Leidinger E. Management of an outbreak of shigellosis in the Vienna Zoo. In: Frolich K, Steinbach F (eds.). Proceedings of the Institute for Zoo and Wildlife Research, Berlin No 766; 2005. p. 164–166.

134. Wolfla CE, Puckett TA. Reversal of neurologic deficit in an adult gorilla with severe symptomatic lumbar stenosis. Spine. 2004;29:E95–E97.

135. World Health Organisation. International Statistical Classification of Diseases and Related Health Problems 10th Revision [Internet]. (cited 2016 July 18) c2010. Available from: http://apps.who.int/classifications/icd10/browse/2010/en

136. Yeo DSY, Lian JE, Fernandez CJ, Lin YN, Liaw JCW, Soh ML, Lim EAS, Chan KP, Ng ML, Tan HC, Oh S, Ooi EE, Tan BH. A highly divergent Encephalomyocarditis virus isolated from nonhuman primates in Singapore. Virol J [Internet]. 2013 [cited 2014 Jul 18];10(1):248. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3750836&tool=pmcentrez&rendertype=abstract

137. Zimmermann N, Pirovino M, Zingg R, Clauss M, Kaup FJ, Heistermann M, Hatt JM, Steinmetz HW. Upper respiratory tract disease in captive orangutans (*Pongo* sp.): prevalence in 20 European zoos and predisposing factors. J Med Primatol [Internet]. 2011 [cited 2014 Jul 11];40(6):365–375. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21770970

138. Zimmermann N, Zingg R, Makara M, Hatt J, Steinmetz H. Computertomographic evaluation of the upper respiratory tract in orang-utans (*Pongo pygmaeus, Pongo abelii*). In: Wibbelt G, Kretzschmar P, Hofer H, Seet S (eds.). Proceedings of the International Conference on Diseases of Zoo and Wild Animals; 2009. p. 102–103.

139. Zoller M, Matz-Rensing K, Hofmann P, Jurek V, Schulz-Schaeffer W, Kaup FJ. Cerebral calcinosis in a young chimpanzee. In: Frolich K, Steinbach F (eds.). Proceedings of the Institute for Zoo and Wildlife Research, Berlin (6); 2005. p. 112–114.

Received for publication 29 October 2015

Appendix 18: Published paper: A retrospective review of western lowland gorilla *(Gorilla gorilla gorilla)* mortality in European zoologic collections between 2014 and 2015. Journal of Zoo and Wildlife Medicine: June 2017, Vol. 48, No. 2, pp. 277-286.

Journal of Zoo and Wildlife Medicine 48(2): 277–286, 2017

A RETROSPECTIVE REVIEW OF WESTERN LOWLAND GORILLA (*GORILLA GORILLA GORILLA*) MORTALITY IN EUROPEAN ZOOLOGIC COLLECTIONS BETWEEN 2004 AND 2014

Victoria Strong, B.Sc. (Hons), B.V.Sc., M.R.C.V.S., Kerstin Baiker, Dipl. E.C.V.P., M.R.C.V.S., Marnie L. Brennan, Ph.D., Dipl. E.C.V.P.H.(PM), M.R.C.V.S., Sharon Redrobe, B.Sc. (Hons) B. Vet. Med., D. Zoo. Med., M.R.C.V.S., Frank Rietkerk, D.V.M., Malcolm Cobb, D.V.C., Ph.D., M.R.C.V.S., and Kate White, Dipl. E.C.V.A.A., D.V.A., M.R.C.V.S.

Abstract: An understanding of the main causes of mortality among captive gorillas is imperative to promoting their optimal care, health, and welfare. A retrospective observational review of mortality among the European zoo–housed western lowland gorilla (*Gorilla gorilla gorilla*) population from 2004 to 2014 was carried out. This is the first published study of mortality in this population. Relevant postmortem data were requested from each collection reporting a death during the study period. Age at death enabled grouping into discrete age categories. Deaths were classified according to cause. The main causes of death overall and for each age category and sex were identified. In total, 151 gorillas from 50 European collections died during the study period. Postmortem data were available for 119 (79%) of the deaths, of which 102 (86%) were classified by cause. Diseases of the digestive system were responsible for most (23%) deaths overall. Also of significance (each accounting for 15% overall mortality) were deaths due to external causes (especially trauma) among young gorillas and cardiovascular disease among adult and aged animals. Being a male gorilla was associated with an 8.77- and 5.40-fold increase in risk of death due to cardiovascular and respiratory disease, respectively. Death due to external causes was 4.45 times more likely among females than males. There was no statistically significant difference in life expectancy between male and female gorillas. The authors conclude that further work is needed to understand risk factors involved in the main causes of death and suggest a need for standardization with regard the approach to postmortem examination and data collection, sample collection, and storage across European zoos.

Key words: Gorilla, gastrointestinal, cardiovascular, captivity, epidemiology, mortality.

INTRODUCTION

The western lowland gorilla (*Gorilla gorilla gorilla*) is identified as critically endangered on the International Union for Conservation of Nature Red List of Threatened Species and is listed in Appendix I of the Convention for International Trade in Endangered Species.[3,9] Endemic to the Central African tropical forests of Equatorial Guinea, Gabon, the Republic of Congo, Angola, Cameroon, and the Central African Republic, the western lowland gorilla is threatened by poaching, the bush-meat trade, infectious disease, and habitat loss.[8]

As for any species whose wild numbers are in dramatic decline, the managed gorilla population is gaining in importance. At the end of 2014, there were 421 western lowland gorillas (190 male, 231 female) housed in 62 zoologic collections across Europe.[18] The European gorilla population is managed under the European Association of Zoos and Aquaria (EAZA) European Endangered Species Programme (EEP) to maintain an outbred and viable self-sustaining population. Matters of gorilla health and welfare are directed by the EEP coordinators and more widely by the Great Ape Taxon Advisory Group and its veterinary advisors.[6] Zoo-housed gorillas act as ambassadors for the species as a whole and play a key role in conservation. They help to create public awareness of the plight of their wild counterparts, raise funds, and provide insurance against extinction. Any threat to their health or welfare is therefore of concern not only to the individual zoologic collection affected but to the future of the species.

Maintaining the good health and welfare status of zoo-housed gorillas requires that individuals tasked with the care of these animals are well informed about the diseases that may develop. This awareness requires regular study of the main causes of illness and death at a population level;

From the School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, United Kingdom (Strong, Baiker, Brennan, Cobb, White); Twycross Zoo, Burton Road, Atherstone, Warwickshire, CV9 3PX, United Kingdom (Strong, Redrobe); and Apenheul Primate Park, J.C. Wilslaan 21, 7313 HK, Apeldoorn, The Netherlands (Rietkerk). Correspondence should be directed to Dr. Strong (ntxvjs@nottingham.ac.uk).

widespread and up-to-date reviews of which are lacking within the literature.[16] Although a review of mortality among the North American captive western lowland gorilla population has been published,[11] no similar reviews for the European population have ever been carried out. The aim of this study therefore was to conduct the first-ever retrospective review of western lowland gorilla mortality between 2004 and 2014 within the EAZA EEP.

MATERIALS AND METHODS

The study was approved by the ethical review committee (July 2013) of the University of Nottingham School of Veterinary Medicine and Science. The study population was defined as including any western lowland gorilla that died on or between 01 January 2004 and 31 December 2014 and was managed under the EAZA western lowland gorilla EEP and housed within a European collection.

Information about the current European zoo–housed gorilla population was generated from studbook data. A report detailing all deaths during the study period was generated using Single Population Analysis and Record Keeping System software.[15] Additional information (clinical notes, animal records, postmortem examination reports) relating to each of the deaths was requested from the holding collection.

Where necessary, the information was translated into English. The raw data were reviewed and key information was extracted. The data fields selected were developed from those used in previous similar studies[7,11] and included the following: animal identification (regional studbook number, name), zoologic collection (at time of death), sex (male, female, undetermined or unrecorded), and date of birth and death. If only a month and year was provided for the date of birth, the date was assumed to be the first of that month. Where only a year was provided the date was assumed to be 01 July of that year. The potential for this assumption to introduce bias into the dataset was explored. The age at death was calculated from the dates of birth and death. The following information was also collected: Was gross postmortem examination data available (yes or no)?; Was histopathology data available (yes or no)?; and What was the main pathology reported to be associated with death (free text)?

Each animal was categorized according to age at the time of death. All animals less than 1 yr of age were classified according to the definitions used for human infant mortality: fetal (stillborn-premature-abortion; never breathed), perinatal-early neonate (breathed, but died aged 0–7 days), and infant (8–364 days).[19] If older than 1 yr, animals were categorized as juvenile until the age of sexual maturity (female, 7 yr; male, 10 yr). They were categorized as subadult-adolescent until the age of dispersal in the wild (15 yr) and as adult thereafter.[11,13] Animals were considered elderly from 35 yr, based upon reports of cessation of female fertility soon after this age.[1]

Deaths were categorized by cause using the International Classification of Diseases system as a guide.[20] Data tables were browsed and screened for missing information or erroneous input before analysis.[17]

Statistical analyses were carried out using Excel (Microsoft, Redmond, Washington 98052, USA) and Prism 6.0d (GraphPad, La Jolla, California 92037, USA). Sex variation in survival and longevity was assessed using log-rank analysis and the Kaplan–Meier method. A Fisher exact test of independence was carried out to assess for sex variation in cause of death. Confidence intervals were set at 95% and P values of <0.05 were considered significant. Data were assessed for normality using a D'Agostino & Pearson omnibus test. Data are expressed as median and range with interquartile range unless stated otherwise.

RESULTS

Data availability and content

Information about the data available for analysis is provided in Table 1. The quality and extent of the postmortem data provided ranged from a single line of information to a comprehensive report detailing the normal and abnormal findings for each organ or body system. Macroscopic postmortem examinations performed ranged from basic external visual inspection by zoo veterinarians to full detailed external and internal examinations by board-certified veterinary or medical pathologists. Histopathology data were available for 93 (84%) of all 111 animals for which gross postmortem data were provided. The organs or samples examined histologically also varied: some institutions carried out histology on samples from every organ, whereas other institutions only carried out histology on samples that appeared grossly abnormal or were clinically suspected to be diseased.

Fetal deaths

Stillbirths and abortions accounted for 22 (15%) (n = 151) deaths, for which data were

Table 1. Information relating to data availability and content.

No. of deaths (*n*)	151
Basic information[a] availability [*n* (%)]	151 (100)
No. of collections (*n*)	50
Response rate [*n* (%)]	44 (88)
Postmortem data availability [*n* (%)]	119[b] (79)
Macroscopic examination data [*n*/119 (%)]	111[c] (93)
Histopathology [*n*/111 (%)]	91 (82)
Exclusions	
Cause not determined [*n* (age category)]	4 (1 adult, 3 infant)
Fetal deaths (*n*)	13
No. categorized by cause [*n* (%)]	102 (86)

[a] Basic information refers to the animal's sex, age, and date of death.

[b] For remaining 32 animals, data were either missing or not provided.

[c] *n* = 8; stillbirths or abortions; macroscopic examination not performed due to autolysis or severe postmortem trauma.

provided for 14 deaths (64%). Of these 14 deaths, macroscopic examination data were available for five (36%) and histopathology data for three (21%). Four of the 14 fetal deaths for which information was available were recorded as abortions, two as stillbirths associated with birth trauma, and seven as stillbirths of unknown cause. Fetal deaths were excluded from age at death and cause of death analysis.

Age at death analysis

In total, 129 animals were subject to age at death analysis. Data were not normally distributed. Median age at death was 21.53 yr (range 0 days–53.81 yr). Figure 1a shows the number of deaths occurring during each 5-yr age interval: mortality was highest in the first 5 yr ($n = 48$). Of these deaths, the majority (81%; $n = 39/48$) occurred within the first 12 mo of life (Fig. 1b); specifically, almost 41% ($n = 16/39$) of these animals were aged 0–7 days at the time of death.

Cause of death analysis

Data from 102 animals underwent cause of death analysis; data were missing for 23 animals and the cause of death was unknown for four animals.

Overall mortality: Diseases of the digestive system were responsible for the largest number of deaths, followed by diseases of the cardiovascular system and death due to external causes (Table 2).

Age-specific mortality by age category: The exact date of birth was unknown for 34 animals because many of these animals arrived in zoos at young but unknown ages in the 1960s and 1970s. For these animals, estimated figures were used. Moving their estimated birth dates to the earliest or latest possible dates would have resulted in recategorization of only three (<9%) of the animals, therefore deeming the potential impact of estimation on the final results to be small.

Differences in the main causes of death between the age categories were observed (Table 3). Deaths occurring due to external causes (especially trauma) were identified as being a particular problem before sexual maturity. In contrast, cardiovascular disease was only identified as a cause of death among adult and aged gorillas. The majority (74%; $n = 17/23$) of the animals dying due to disease of the digestive system were also classified as adult or aged, although deaths within this category were reported across the ages (range 10 days–51.63 yr).

Sex-specific mortality

The median age at death for males (41%; $n = 62$) was 21.41 yr (range 0 days–51 yr) and for females (54%; $n = 81$) was 21.41 yr (range 0 days–52 yr). Age at death (survival) data were used to generate a Kaplan–Meier curve (Fig. 2). Although the curve shape implied a slight reduction in survival of males during the early years and again later in life, log-rank (Mantel–Cox test) analysis showed no statistically significant difference in survival between the sexes overall ($P = 0.3886$) or during any of the age intervals tested (<15, >15, >30, and >35 yr).

In females the three main causes of death were disorders of the digestive system (28%), external causes (21%), and neoplastic disorders (12%). Among males, the three main causes of death were diseases of the circulatory (30%), respiratory (19%), and digestive (16%) systems.

The number of deaths due to each cause, divided by sex, can be seen in Figure 3. A Fisher exact test of independence found that sex variation in cause of death was statistically significant for diseases of the circulatory system (males > females; relative risk [RR] = 8.77; confidence interval [CI] = 2.375–33.68; $P = 0.003$) and respiratory system (males > females; RR = 5.40; CI = 1.375–21.75; $P = 0.017$) and for death due to external causes (females > males; RR = 4.45; CI = 1.208–17.29; $P = 0.0225$). Although females seemed to be overrepresented

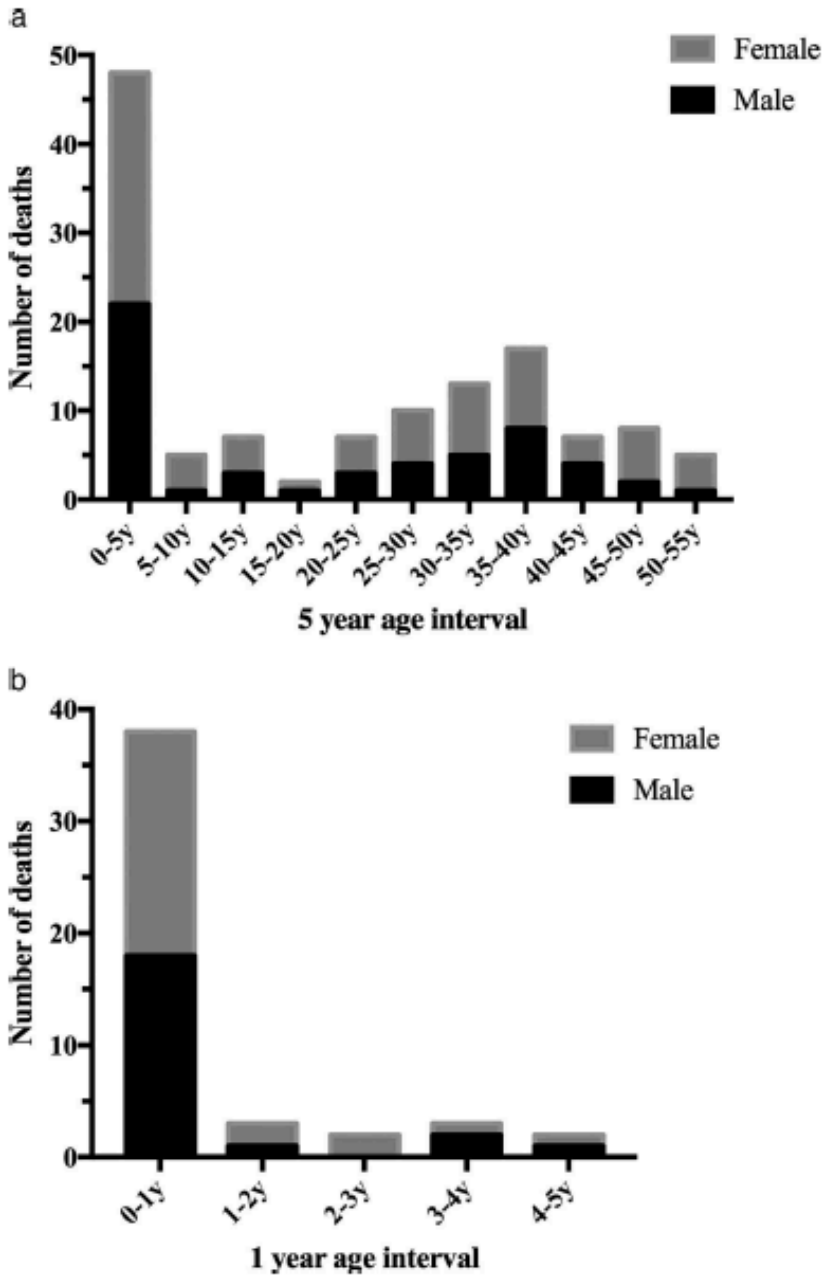


Figure 1. **a.** Number of deaths per 5-yr age (at death) interval, divided by sex. **b.** First 5-yr mortality: number of deaths per 1-yr age (at death) interval, divided by sex.

among animals dying due to digestive system and neoplastic disorders, these differences were not statistically significant. All animals dying due to disease of the genitourinary system (n = 6) were female.

DISCUSSION

This review identified great variation between zoologic institutions with respect to the frequency or consistency with which macroscopic postmortem examination, histopathologic ex-

Table 2. Overall mortality associated with each cause of death.[a]

Cause of death category	Overall mortality (%)	Age at death			Diseases and pathologies reported (*n*)
		Median	IQR[b]	Range	
Diseases of the digestive system	23 (23/102)	30.06 yr	22.36 yr	10 days–51.63 yr	Peritonitis due to gastrointestinal inflammation (bacterial, protozoal) with or without perforation (12) Gastrointestinal inflammation (enteritis, colitis) (8) Gastrointestinal bloat (1) Dental disease (1) Necrotizing esophagitis (1)
Diseases of the circulatory system	15 (15/102)	35.12 yr	9.06	17.93–44.71 yr	Myocardial fibrosis (5) Aortic aneurysm or rupture (3) Heart failure (3) Dilatation (2) Hypertrophy (2)
External causes	15 (15/102)	1.06 yr	0.49 yr	0 days–6.89 yr	Conspecific trauma (12) Maternal neglect (2) Accidental death (1)
Disorders of the respiratory system	10 (10/102)	2.74 yr	24.16 yr	12 days–40.75 yr	Bronchopneumonia/pneumonia (often bacterial) (7) Pneumococcal pleurisy (1) Upper respiratory tract inflammation and obstruction under anesthesia (1)
Conditions arising in the perinatal period	10 (10/102)	0 days	0 days	0–6 days	Fetal immaturity (2) Infection (2) Amniotic fluid aspiration (2) Hypoxia (1) Generalized weakness, failure to thrive, undetermined (3)
Infectious diseases (not included elsewhere)	9 (9/102)	13.20 yr	23.53 yr	317 days–48.80 yr	Parasitic (4) including *Echinococcus multilocularis*, *Echinococcus granulosus*, and *Ballamuthia mandrillaris* Viral (3) including *Varicella zoster*, *Herpes simplex* virus, simian T-cell leukemia virus–associated chronic illness Bacterial (2) including *Escherichia coli* and *Brucella*-like organism
Neoplastic diseases	8 (8/102)	37.44 yr	11.50 yr	3.96–50.21 yr	Metastatic neoplasia of uterus (5) Mammary glands (1) Various organs (no histologic diagnosis) (1)
Diseases of the genitourinary system	6 (6/102)	29.73 yr	9.76 yr	9.76–38.54 yr	(Pyelo-)nephritis (3) Perinephric abscess (1) Peritonitis, secondary to ruptured ovarian cyst (1) Renal failure (1)

[a] Information relating to the age of the animals in each category and a list of reported diseases and pathologies is provided. Animals dying due to other causes (neurologic, $n = 2$; musculoskeletal, $n = 1$; endocrine, $n = 1$; pregnancy and parturition, $n = 1$; miscellaneous, $n = 1$) each account for <2% of all deaths and are not included in the table.

[b] IQR indicates interquartile range.

Table 3. Cause of death by age category.[a]

Age category	No. of deaths	No. categorized according to cause of death (M/F/U)[b]	Main cause of death [*n*; % (M/F/U)]		Other causes (*n*)
Perinatal, 0–7 days	16	14 (6/7/1)	Perinatal	10; 71% (6/4/0)	External causes (4)
Infant, 8–364 days	23	17 (9/8/0)	External causes	8; 47% (2/6/0)	Respiratory (4) Digestive (4) Infectious (1)
Juvenile, 365 days–7 yr (F); 365 days–10 yr (M)	11	7 (1/6/0)	External causes	3; 43% (0/3/0)	Respiratory (2) Digestive (1) Neoplastic (1)
Subadult or adolescent, 7–15 yr (F); 10–15 yr (M)	10	8 (2/7/0)	Infectious disease	4; 50% (1/3/0)	Digestive (1) Genitourinary (1) Respiratory (1) Pregnancy/parturition (1)
Adult, 15–35 yr	32	26 (12/14/0)	Digestive system	10; 38% (2/9/0)	Circulatory (7) Genitourinary (3) Infectious (2) Endocrine/metabolic (1) Neoplastic (1) Neurological (1) Respiratory (1)
Aged or elderly, >35 yr	37	30 (13/17/0)	Circulatory system	8; 27% (6/2/0)	Digestive system (7) Neoplastic (6) Genitourinary (2) Infectious (2) Respiratory (2) Neurological (1) Miscellaneous (1) Musculoskeletal (1)

[a] Number of deaths occurring and the causes of mortality for each age category are shown. For each age category the total number of deaths is displayed.

[b] M indicates male; F, female; U, unknown or undetermined.

amination, or both were performed; organs examined; samples collected; additional diagnostic tests performed; level of expertise of the individual performing the examination; quality and detail of the written report; and the vocabulary and terminology used in the pathologic descriptions and diagnoses. Standardization of postmortem data collection and analysis as well as centralization of data recording and storage across the western lowland gorilla EEP would facilitate future studies of this type and allow for not only ongoing monitoring of mortality within this population, but also comparison with other great ape taxa and their wild counterparts. Ideally, deaths should be coded at the time of occurrence and entered into the database to limit the potential for bias to be introduced at a later date. The authors recommend the system used for this review (adapted from the International Statistical Classification of Diseases and Related Health Problems-10 classification system).

Diseases of the digestive system were found to be the main cause of mortality, and diseases of the circulatory system and death due to external causes were also identified as being of great significance. The findings from this study are consistent with those of a similar review among the North American zoo–housed gorilla population.[11] However, they differ from the findings of a review of mortality among the British (Irish) zoo–housed gorilla population from 1896 to 2005.[7] In this latter study, gastrointestinal disease was reported to be on the decline, as it was responsible for only 15% mortality and only the third most common cause of death behind respiratory (27%) and multisystem (26%) diseases. Interestingly, cardiovascular disease was also identified as the cause of death in only 8.9% of animals in this study. The discrepancies in these datasets might imply key differences in the disease risk factors in North American, European, and British (Irish) subpopulations. However, closer examination of the raw data revealed marked differences in the

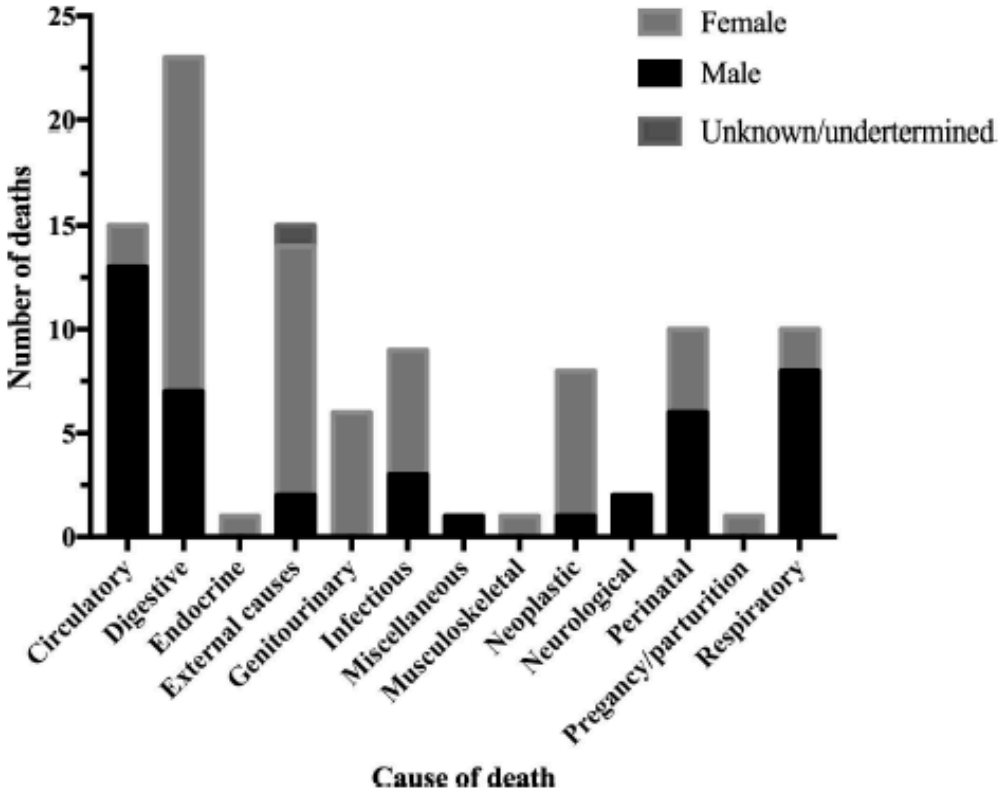


Figure 2. Kaplan–Meier curve comparing survival (age at death) of male and female gorillas within the study population (only those animals aged 1 day or older were included).

categorization systems used, which might have resulted in under- or overrepresentation of certain disease categories. In addition, the study periods varied greatly (pan-European study 2004–2014, North American study 1980–1994; British (Irish) study 1896–2005). It is possible that the accuracy of diagnoses recorded historically are less than those recorded more recently, due to advances in veterinary diagnostic capabilities. It is possible however that the discrepancies reflect a genuine shift in disease pattern over time, of which there is evidence in the literature; historically, infectious diseases were the predominant cause of death.[2,4,5] Dramatic changes to animal husbandry over recent years, along with advances in veterinary medicine, have resulted in vast improvements to animal health. Subsequently, infectious diseases are declining and age-related and chronic diseases are becoming increasingly prominent.[10] Despite the pattern of increasing captive longevity, the highest proportion of deaths in this study population still occurred within the first 5 yr of life, a conclusion also drawn >30 yr ago.[5] The most common cause of death in animals in this age group was external causes, usually trauma by conspecifics, and similar findings were reported among the North American population.[11] Any attempts at reducing mortality within this group of animals must therefore be focused more around management practices than veterinary intervention.

Peritonitis was identified as the cause of death in 14 (11 female, 3 male) animals under study, with more than half ($n = 12/23$) of those in the "digestive system disorders" category. Intra-abdominal abscesses have also previously been identified as a significant problem among female gorillas in the North American zoo–housed population.[12] The causes of these abscesses or adhesions can be organ perforation, enteritis, reproductive tract infection, iatrogenic causes, and nutritional deficiencies. The careful dissection of abscesses or adhesions during postmortem examination is therefore very important to ensure the accurate description of lesions and correct classification of the cause of death.

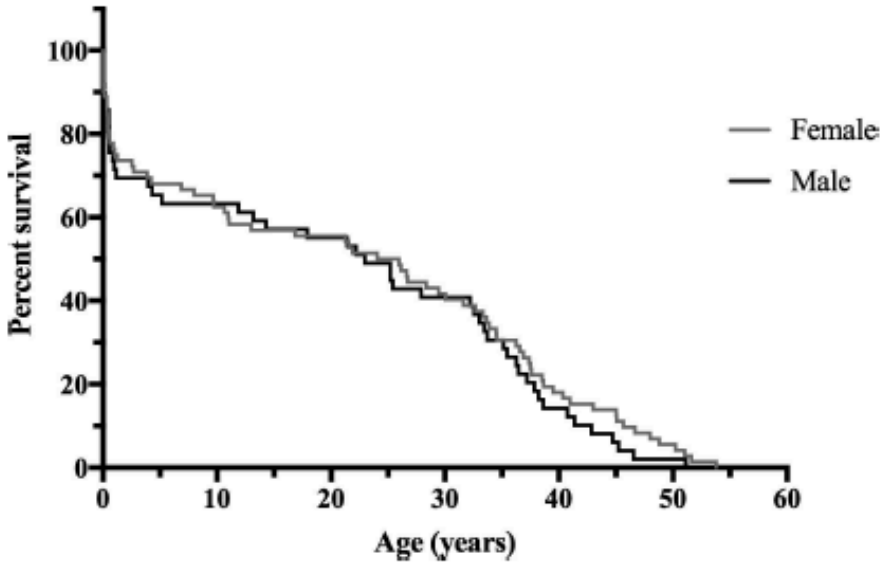


Figure 3. Column chart showing the number of deaths for each age category, divided by sex.

Additional work is required to investigate the epidemiology of the main causes of death among zoo-housed gorillas to identify trends, causes, predispositions, and risk factors. Of interest for future investigation would be the role that factors such as infectious agents, environmental or husbandry factors, diet, sex, genetics, and stress might play, especially in the development of both digestive and cardiovascular disorders. In addition, the high incidence of digestive system disease as a cause of mortality suggests a need for proactive management on an individual collection level, for example, through routine fecal screening for parasitic or bacterial infections, fecal consistency scoring, stress reduction, and immediate treatment of conditions as they arise. The high numbers of deaths caused by cardiovascular disease, particularly among adult and aged males, suggests a need for proactive screening of animals to identify affected animals and for the treatment of affected individuals to help reduce mortality, prolong life span, or both. Collection and storage of potentially useful biologic samples of interest would help to facilitate future investigation into these diseases.

There is a critical need for similar reviews to be published for the remaining three great ape taxa. Retrospective studies of mortality among these populations will be informative for the clinical management, husbandry, and care of these animals in captivity. In addition, comparisons of the findings for each of the four taxa and between them and their wild counterparts are likely to be informative in identifying differences and similarities in the disease risk factors to which they are exposed.

Limitations of the study

Raw data collection: Although EAZA guidelines for postmortem examination and sample collection are available, there is currently no compulsory protocol for the completion of postmortem exams or reports in EAZA Zoos. Autolysis and carcass decay prevented informative examination in 7% of the animals under study. However, because all of these deaths were fetal deaths (abortions or stillbirths), which were not included in the cause of death data analysis, the effect of this missing data on the final results is likely to have been negligible.

This study was a retrospective, multicenter, observational study. The retrospective and observational nature of this study meant that raw data recording was not designed specifically for purpose. The authors therefore had no control over the methods of data collection, recording system, or terminology used. To a certain extent, this has the potential to affect the reliability of the findings, but it is an issue inherent to all studies of this type and is largely unavoidable.[14] In an effort to minimize any potential impact that this might have upon the final results, complete raw datasets (full postmortem reports) as opposed to

mere statements relating to the animals' cause of death were obtained where possible and reviewed in detail.

Data processing: There was a degree of ambiguity with respect to cause of death category for some animals, especially those with multiple equally severe or extensive coexisting pathologies on postmortem examination. In addition, categorization for the purposes of quantitative analysis can result in loss of detail. This is particularly true for those deaths for which the cause of death is ill-defined, for example, in cases of perinatal or perianesthetic death. Further understanding of the actual pathologies that resulted in the animals' death is needed for more in-depth epidemiologic elucidation of risk factors and ultimately of prevention.

Data analysis and results: In this study, data from 151 deaths in total were included, making it the largest study of its type to date. In addition, the multicenter nature of this study and the excellent participation rate (88%) achieved means that the data collected can be considered representative of the wider population. The recent time period studied also deems the findings up to date and relevant. However, the statistical significance of some of the results should be interpreted with caution considering the size of the dataset. For example, 95% CIs reported for sex variation in cause of death were very wide. Greater certainty about the role that sex might play in disease risk requires study of a larger number of animals.

CONCLUSIONS

This review concluded that further work is needed to

- Standardize and improve the approach to postmortem examination, data collection, processing, storage, and analysis across European zoologic collections (ideally facilitated by the Gorilla EEP)
- Understand the associated risk factors to reduce the number of deaths occurring due to gastrointestinal and cardiovascular diseases among adult and aged animals
- Investigate the observed sex variation in causes of death
- Reduce and manage the number of deaths occurring among young gorillas to limit the potential for long-term effect on overall population sustainability
- Compare patterns of mortality observed in this population with those of their wild counterparts and of other great ape taxa held in zoos.

This review is intended to serve as a model for future mortality studies and to lay the foundation for the ongoing monitoring of patterns of mortality within this and other populations. The key ambition of regular mortality review is to inform future management practice and policy and ultimately the enhancement of the health and welfare of the animals concerned.

Acknowledgments: The authors thank all committee members of the European Association of Zoos and Aquaria Great Ape Taxon Advisory Group and acknowledge staff and colleagues at the named institutions for assistance and support. Finally, thanks also go to Wendela Wapenaar and others for translational assistance and to Jess French for early work on this project. This project forms part of the work conducted by the Ape Heart Project, based at Twycross Zoo and in fulfilment of the doctoral thesis of VS.

LITERATURE CITED

1. Alberts SC, Altmann J, Brockman DK, Cords M, Fedigan LM, Pusey A, Stoinski TS, Strier KB, Morris WF, Bronikowski AM. Reproductive aging patterns in primates reveal that humans are distinct. Proc Natl Acad Sci U S A 2013;110:13440–13445. Available from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3746941&tool=pmcentrez&rendertype=abstract.

2. Benirschke K, Adams FD. Gorilla diseases and causes of death. J Reprod Fertil Suppl. 1980;Suppl 28:139–148.

3. Convention on International Trade in Endangered Species. Convention on International Trade in Endangered Species of wild fauna and flora: appendices I, II and III [Internet]. 2015. Available from http://www.cites.org/sites/default/files/eng/app/2015/E-Appendices-2015-02-05.pdf

4. Cousins D. Diseases and injuries in wild and captive gorillas: *Gorilla gorilla*. Int Zoo Yearb. 1972;12(1):211–218.

5. Cousins D. Mortality factors in captive gorillas (*Gorilla gorilla*). Int Zoo News. 1983;30:5–17.

6. European Association of Zoos and Aquaria. European Association of Zoos and Aquaria [Internet]. Collection planning. 2013 [cited 2013 November 28]. Available from http://www.eaza.net/activities/cp/Pages/Collection Planning.aspx

7. Hewitt S. Preliminary findings of the prevalence of diseases in captive western lowland gorillas (*Gorilla gorilla gorilla*) in the United Kingdom and Ireland (1896–2005). In: Proc 7th Ann Symp Zoo Res, Twycross Zoo, Warwickshire, United Kingdom, 7–8 July 2005. p. 222–231.

8. International Union for Conservation of Nature. Regional action plan for the conservation of western lowland gorillas and central chimpanzees 2015–2025. Gland, Switzerland: International Union for Conservation of Nature Species Survival Commission Primate Specialist Group; 2015.

9. International Union for Conservation of Nature. The International Union for Conservation of Nature red list of threatened species. Version 2016-1 [Internet]. 2016 [cited 2016 August 16]. Available from http://www.iucnredlist.org/

10. Janssen DL, Bush RM. Review of the medical literature of great apes in the 1980s. Zoo Biol.1990;9: 123–134.

11. Meehan TP, Lowenstine LJ. Causes of mortality in captive lowland gorillas: a survey of the SSP population. Proc Am Assoc Zoo Vet; 1994. p. 216–218.

12. Mylniczenko ND. A preliminary report on intra-abdominal abscesses in captive western lowland gorillas (*Gorilla gorilla gorilla*). In Proc Am Assoc Zoo Vet; 2003. p. 62–66.

13. Rowe N. The pictorial guide to the living primates. East Hampton, New York: Pogonias Press; 1996.

14. Senior JM. Morbidity, mortality, and risk of general anesthesia in horses. Vet Clin North Am Equine Pract. 2013;29:1–18. Available from http://www.ncbi.nlm.nih.gov/pubmed/23498043

15. Single Population Analysis and Records Keeping System. Species 360 formerly known as ISIS (ISIS). Eagan, Minnesota: Single Population Analysis and Records Keeping System version 1.5; 2005.

16. Strong V, Grindlay D, Redrobe S, Cobb M, White K. A systematic review of the literature relating to captive great ape morbidity and mortality. J Zoo Wildl Med. 2016;47(3):697–710.

17. Van den Broeck J, Argeseanu Cunningham S, Eeckels R, Herbst K, Armitage P, Berry G, Ki F, Liu J, Wang W, Chow S, Horn P, Feng L, Li Y, Pesce A, Hadi A, Altman D, Snedecor G, Cochran W, Iglewicz B, Hoaglin D, Hartigan J, Hartigan P, Welsch R, Haykin S, Myers R, Wainer H, Schachts S, Wang R, Lauritsen J, Bruus M, Myatt M, Bauer U, Johnson T, Winkler W, West M, Winkler R, Gardner M, Altman D, Fergusson D, Aaron S, Guyatt G, Hebert P, Allison P, Twisk J, Vente W de, Schafer J, Gonzalez M, Ogus J, Shapiro G, Tepping B. Data cleaning: detecting, diagnosing, and editing data abnormalities. PLoS Med. 2(10):e267. Available from http://dx.plos.org/10.1371/journal.pmed.0020267

18. Wilms T. International studbook for the western lowland gorilla (*Gorilla g. gorilla*) (Savage & Wyman, 1847). Frankfurt, Germany: Frankfurt Zoo; 2010.

19. World Health Organization. Neonatal and perinatal mortality: country, regional and global estimates. Geneva, Switzerland: World Health Organization; 2006.

20. World Health Organization. International statistical classification of diseases and related health problems, 10th revision [Internet]. 2010. Available from http://apps.who.int/classifications/icd10/browse/2010/en

Accepted for publication 27 December 2017

Appendix 19: Abstract: A comparison of cardiovascular effects of two different anaesthetic protocols in chimpanzees (*Pan troglodytes*). Association of Veterinary Anaesthetists Spring Meeting, Manchester, United Kingdom. 26-28th April 2017

Victoria Strong[1,2]; Torsten Möller[3]; Ann-Sofie Tillman[4]; Stefan Träff[4,5]; Louise Guevara[3]; Mike Martin[7]; Sharon Redrobe[2]; Malcolm Cobb[1]; Kate White[1]

1. School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus
2. Twycross Zoo, Burton Road, Atherstone, Warwickshire, CV9 3PX, United Kingdom
3. Kolmården Wildlife Park, 618 92, Kolmården, Sweden
4. Diagnostic Centre, Vrinnevi Hospital, Norrköping, Sweden
5. Heart Centre, University of Linköping, Sweden
6. Willows Veterinary Centre and Referral Service, Highlands Road, Shirley, Solihull, B90 4NH, United Kingdom

The use of alpha 2 agonists in great apes undergoing anaesthesia for cardiovascular evaluation is controversial. This pilot study aimed to evaluate the effect of medetomidine in healthy chimpanzees.

Six chimpanzees (*Pan troglodytes*) (4 to 16 years old; 45.6 ± 22.7 kg; 2 males, 4 females) were anaesthetized on two occasions in a cross over design. All animals were offered midazolam (1 mg kg^{-1}) prior to anaesthesia. Anaesthesia was induced with zolazepam/tiletamine (3-4 mg kg^{-1}) (ZT) or zolazepam/tiletamine (2 mg kg^{-1}) and medetomidine (0.02 mg kg^{-1}) (ZTM), and maintained with intermittent boluses of ketamine (IV) or zolazepam/tiletamine (IM).

Quality of induction, time to recumbency, number of supplemental boluses, anaesthesia quality and recovery characteristics were recorded. Chimpanzees were continuously monitored and HR, PR, *f*r, SpO_2, SAP, DAP, rectal temperature, mucous membrane colour recorded. Animals underwent a 12 channel ECG, full haematology, biochemistry and cardiac biomarker assessment. A detailed echocardiographic examination (Ao, LA, IVSd, LVIDd/s, LVP, EF, LVOT, $Vel_{PV/AV}$, $Vel_{E/A}$) was carried out by the same blinded observer. Data were compared using Student's paired t-test or Wilcoxon rank tests as appropriate.

During ZTM procedures HR, PR, SAP and DAP were significantly lower. There were no significant differences in the echocardiographic measurements between the two protocols. Quality of anaesthesia was significantly better with ZTM and no additional boluses were required. ZT protocol required multiple 'top ups'.

Both combinations are suitable for immobilization and cardiovascular evaluation of healthy chimpanzees. Further work is required to evaluate the effect of medetomidine in cardiovascular disease.

The study was performed with the approval of the ethical review committee of the University of Nottingham's School of Veterinary Medicine and Science (February 2016, 1692 160222).