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M.Sc. Conservation Science

Necropsy Reports from the European Captive Population of the Amur Leopard (*Panthera pardus orientalis*):

as an example of zoos' contribution to inform captive population managers and support conservation activities

By Samantha Earle

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science and the Diploma of Imperial College London

September 2008

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Abstract

A morbidity and mortality survey of the European captive breeding programme of the Amur leopard (*Panthera pardus orientalis*) (AL-EEP) is crucial to assess health of the population to (1) improve husbandry and management, (2) contribute to a disease management strategy for the proposed reintroduction from AL-EEP stock. A comprehensive mortality survey requires good quality necropsy reports from deaths in the population. The subject of this thesis is to (1) determine the extent to which zoos are meeting their obligation to conduct necropsy examinations and provide good quality necropsy reports, (2) to conduct an elementary mortality survey to assess whether zoos have been providing enough information to inform captive population managers and conservation strategies. All zoos that have ever held Amur leopards (AL) were requested to submit all necropsy reports to the AL-EEP veterinary advisor. Necropsy examinations were conducted on at least 51% of deaths, but necropsy reports were written for 37% deaths. Since the first death of AL in captivity in 1969, there has been no significant change in the proportion of deaths for which there has been a necropsy report; but there has been a significant improvement in the quality of the necropsy reports produced. The overall behaviour of zoos in producing necropsy reports is independent of one another as they do not exhibit a similar trend of report writing over time. The number of deaths at each zoo may partially explain whether a zoo conducts a necropsy and writes a report, although a quantitative analysis of frequencies is too simplistic. Social, economic and political situations of each zoo at the time of an AL death are far more likely to explain the extent of necropsy examinations and good quality necropsy reports written. The most frequent causes of death were identified and their prevalence (1) over time, (2) amongst zoos, and (3) with age at death were investigated. The elementary mortality survey demonstrates that mortality information provided by zoos can be informative to captive population managers and populations directly involved with conservation strategies of species in the wild. However, across European zoos there is a substantial shortfall in the extent to which necropsy examinations are being conducted and the quality of the reports provided. Further investigations to explain and then improve zoo behaviour to collect and provide good quality information are suggested.

Acknowledgements

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List of Acronyms and Abbreviations

AL	Amur leopard
AL-EEP	European captive breeding programme of the Amur leopard
ANOVA	Analysis of Variance
ARKS	Animal Record-Keeping System
CoD	Cause of Death
DP	Disease Process
EEP	European Endangered Species Programme
GLM	Generalised Linear Model
ISIS	International Species Information System
IUCN	International Union for the Conservation Nature and Natural Resources
J.L.	Dr. John Lewis – veterinary advisor to the European captive breeding programme of the Amur leopard, and veterinary advisor to the Amur leopard reintroduction programme
MedARKS	Medical Animal Record-keeping System
M.S.	Dr. Mark Stidworthy - veterinary pathologist to the European captive breeding programme of the Amur leopard
PMP	Population Management Programme – American captive breeding programme
S.C.	Sarah Christie – co-coordinator of the European captive breeding programme of the Amur leopard
T.A.	Tanya Arzhanova - co-coordinator of the European captive breeding programme of the Amur leopard
ZIMS	Zoological Information Management System

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1. Introduction

1.1. The Role of Zoos in Conservation

In recent times zoos have re-positioned themselves from entertainment centres to conservation organisations (West and Dickie 2007). There are various mean that *ex-situ* breeding programmes can contribute to conservation, such as captive breeding for the purposes of reintroduction, conservation education, using animals as fundraising ambassadors for conservation organisations a, development of technologies and research relevant to *in-situ* conservation (Conway 2003).

Reintroduction of captive stock to an area within recent historical range from where a species has been extirpated is becoming an increasing popular conservation strategy (Seddon *et al.* 2007). Amongst zoos there has been a vast uptake to the captive breeding (Stanley Price 2005; Stanley Price and Fa 2007). However, historically reintroduction attempts have a low success rate (Seddon 1999) and are viewed by some as 'wasteful of conservation funds' and little more than 'romanticised schemes' (Rahbek 1993). There are various reasons why a reintroduction attempt may, such as lack of financial investment, poor post-release monitoring and evaluation, and disease (Seddon *et al.* 2007). Every effort must be made to increase the success rate of reintroductions, zoos that supply animals for release have potential to contribute to developing reintroduction techniques. However, Baker (2007) concludes that many zoos have yet to go beyond maintenance of a captive population, and are not managed in ways to ensure that they contribute as much as they could. There has been no effort to evaluate the true contribution that zoos are making to reintroduction strategies or conservation activities as a whole (Baker 2007).

1.2. Conservation of the Amur Leopard

1.2.1. Reintroduction as a Conservation Strategy

The rapid loss of global biodiversity is well documented (Pimm *et al.* 1995). Conservation comprises of actions that attempt to reverse or decrease the rate of loss of biodiversity (Leader-Williams *et al.* 2007). As a part of ecosystem restoration species reintroduction is an increasingly popular conservation strategy (Griffith *et al.* 1989; Kleiman *et al.* 1994; Cunningham 1996). Reintroduction is defined as "an attempt to establish a species in an area which was once part of its historical range, but from which it has been extirpated or gone extinct" (IUCN 1995).

An increasing number of animal reintroduction projects rely on captive born individuals to provide release stock (de Boer 1994). In addition, as zoological institutions (hereafter referred to as 'zoos') move away from entertainment centres to embrace conservation activities (West and Dickie 2007), there has been a surge of interest in captive breeding endangered species with a view to direct involvement in reintroduction projects (Wilson and Stanley Price 1994; Stanley Price and Fa 2007). One such project is the proposed reintroduction of the Amur leopard (*Panthera pardus orientalis*) into the Russian Far East, which has been reduced to a single population of 25-44 individuals (Miquelle *et al.* 1996; Aramilev *et al.* 1998; Pikunov *et al.* 1999; Uphyrkina *et al.* 2002; Kostyria *et al.* 2003; Miquelle and Murzine 2003)..

1.2.2. Disease Impacts on Reintroduction

Small populations are particularly vulnerable to the impact of disease (Ballou 1993; Viggers *et al.* 1993; Woodford 1993; Woodford and Rossiter 1994; Cunningham 1996; Snyder 1996; Mathews *et al.* 2006; Kock *et al.* 2007). In reintroduction programmes released individuals may be at risk of exposure to disease-causing agents present in the release area. Conversely the introduction of disease from reintroduced individuals may not only compromise the establishment of the target species, but also have detrimental effects on wildlife in the same site (Viggers *et al.* 1993; Cunningham 1996; Kock *et al.* 2007). During planning and preparation for any reintroduction project, it is crucial that a disease management strategy is developed to minimise these risks (Ballou 1993; Cunningham 1996).

1.2.3. Health Assessment of Captive Stock

Zoos bear a substantial responsibility to maintain healthy populations and provide veterinary information of individuals for assessment of the health of the population by veterinarians (Lewis 2007). Morbidity (relating to illness) and mortality (relating to death) surveys of captive populations are an essential component for responsible captive management of any species (Munson 1991).

The importance of a health assessment is heightened when a captive population will provide a reintroduction programme with stock for release into the wild – such as that of the Amur leopard European Endangered species Programme (AL-EEP). Analysis of the health of a captive population prior to selection for release is a prerequisite for any reintroduction programme (reasons explained in section 2.2) and continues to form a major component of the selection criteria for reintroduction candidates (Viggers *et al.* 1993; Cunningham 1996; Snyder 1996).

A population manager is reliant upon a) zoos collecting morbidity and mortality data from clinical and necropsy examinations[1], b) writing a necropsy report to summarise the observations and findings, and c) submitting the report to population managers to assess the health of the population. Although Munson and Cook discuss the problems and limitations of gathering morbidity data for captive populations (Munson and Cook 1993), there is little discussion of the behaviour of zoos to collect and submit data captive population managers and to contribute to conservation strategies of the same species in the wild.

1.2.4. Record-keeping Software

In principal standard international animal medical record keeping software (MedARKS) is available to collate veterinary data from the worlds' zoos. This aim is often frustrated by the general difficulties of data entry and the lack of resources available for data input; resulting in varied quantity and quality of the data available. In addition, extraction of information for analysis is challenging, hindering research attempts by interested parties, such as captive population managers (pers. comm. J.L.).

The next generation of international animal record-keeping software, Zoological Information Management System (ZIMS), is in development. Completion of ZIMS will be a real-time global database that will be able to record and share data on a comprehensive range of aspects concerning captive populations, such as enclosure design, diet and *veterinary information* (ISIS 2008). In principal ZIMS will meet the requirements of captive population managers. However, the development of ZIMS is a few years behind schedule, and even when it becomes available uptake will not be universally instant (pers. comm. J.L.). There is an urgent need for veterinary data to inform the imminent reintroduction of the AL.

1.3. Problem Statement

Zoos have a responsibility to provide veterinary data to captive species population managers for the purpose of effective management to maintain a healthy population. A health assessment of the European captive population of Amur leopard (AL-EEP) is vital as a reintroduction from this population is planned to start within the next two to three years. Morbidity and mortality surveys form a key component of a health assessment but adequate data is not available from global animal record-keeping software. It is not known whether zoos have been conducting necropsy examinations for every AL death, recording observations of the prevalence of disease in a necropsy report and then

[1] *A necropsy is an' examination of the internal structures of the body performed after death' which determines the cause of death and evaluates the presence of disease. WEST, G. P. (Eds.) (1975)* Black's Veterinary Dictionary, *London, Adam & Charles Black.*

submitting the data to captive population managers to provide sufficient data for a comprehensive mortality survey. If there is a substantial lack of data there needs to be a better understanding of the factors that limit the collection and recording mortality data by zoos, in order that solutions can be implemented so that more necropsy examinations are conducted, more good quality reports written.

1.4. Application of the Thesis

1.4.1. Application of the Thesis to a Research Perspective

A bespoke database for the AL-EEP developed as part of this thesis will be distinct in its approach to collection of veterinary data from a captive population. The collation of available veterinary data of a population into a bespoke database will serve as:

- a source of information about each individual in the population, whether dead or alive;
- storage for all veterinary data from which there can be analysis of morbidity and mortality within the population;
- to enable comparisons of data with other species to determine if the Amur leopard suffers from the usual problems of other captive cat species;
- a means through which to generate normal biological data such as haematology and serum biochemistry values for the taxon;
- an information resource detailing the locations and type of all available bio-samples to facilitate future research efforts – at present financial, and in some cases, technical issues restrict investigations into the most essential parameters.

This thesis will be the first known assessment of the behaviour of zoos in supporting conservation activities. The collection and submission of mortality data of the AL-EEP in this thesis is used as an example of the behaviour of zoos. As a first assessment of this nature, it is hoped that the results of this thesis will guide future research into factors that determine the behaviour of zoos across Europe.

As the first, albeit brief, mortality survey of the AL-EEP and the database will provide the mechanism for future research of the health of the population.

1.4.2. Application of the Thesis to a Policy Perspective

It is hoped that the database developed as part of this thesis will provide a model that can be distributed for use in the management of veterinary information of captive populations of other species. The author acknowledges that there will be areas of the database design that will require improvement, nonetheless it is hoped that this version is first step towards the creation of a useful tool that can be distributed to other captive population managers.

Zoos within the AL-EEP have a responsibility to provide veterinary data for an assessment of the health of the population. Although little mortality information of the captive Amur leopard population is currently available from official record-keeping software, it should not be assumed that the data has not been collected in the first instance. Only after direct communication with zoos to request submission of mortality data will be possible to determine how much information is available for assessment. If zoos are not meeting their obligations to provide morbidity information the thesis will investigate what influences the behaviour of zoos to collect and submit morbidity data and how to promote the collection and submission of veterinary data from zoos in the AL-EEP.

The results of the mortality survey will inform captive population managers of disease prevalence over time, between zoos and in cats of different age at death. A review of husbandry and veterinary activities can ensure that a healthy population is managed in the long-term.

1.5. Aims, Objectives and Hypotheses

1.5.1. Aims

The first aim of this thesis is to establish whether zoos in the AL-EEP have been meeting their obligations to collect mortality information and provide good quality necropsy reports from all deaths in the AL-EEP (refer to section 3.3.3. for a definition of a good quality report). The second aim is to conduct an elementary mortality survey of the AL-EEP from what information is available. In order that data from reports can be analysed there needs to be a standardisation and pooling of information into a bespoke database. Therefore, a component of this thesis is concerned with the design and development of a database for such a purpose.

1.5.2. Objective A: Extent of Necropsy Examinations and Necropsy Report Writing

- Are zoos conducting necropsy examinations and how are observations being recorded?
- Are zoos meeting their obligation to write necropsy reports and then to submit them to the veterinary advisor?
- Has the proportion of deaths for which there is a necropsy report changed over time?

- Are some zoos more compliant than others? Which zoos have a poor compliance rate and require further investigation?
- Is the compliance between zoos similar over time?

Hypotheses:

A. Over time, there has been an increase in the proportion of deaths for which a necropsy report is written. *Null = over time, there is no difference in the proportion of deaths for which a necropsy report is written.*

B. Between zoos there is a significant difference in the proportion of deaths from which there has been a necropsy report. *Null = between zoos there is no significant difference in the proportion of deaths for which there are necropsy reports.*

C. Over time, individual zoos exhibit a similar pattern in the proportion of deaths for which a necropsy report is written. *Null = over time, individual zoos do not exhibit a similar pattern in the proportion of deaths for which a necropsy report is written.*

1.5.3. Objective B: Quality of Necropsy Reports

- Of what quality are necropsy reports in the AL-EEP?
- Has the quality of necropsy reports improved with time?
- Do some zoos produce better quality necropsy reports than other?
- Amongst zoos is there a similar pattern in the quality of necropsy reports over time?

Hypotheses:

D. Over time there is a significant increase in the proportion of necropsy reports with a conclusive cause of deaths. *Null = Over time there is no significant increase in the proportion of necropsy reports with a conclusive cause of death.*

E. Between zoos there is a significant difference in the proportion of necropsy reports for which there are a conclusive cause of deaths. *Null = between zoos there is no significant difference in the proportion of necropsy reports for which there are conclusive causes of death.*

F. Over time, individual zoos report exhibit a similar pattern in the proportion of necropsy reports with a conclusive cause of death. *Null = over time zoos do not exhibit a similar pattern in the proportion of necropsy reports with a conclusive cause of death.*

1.5.4. Objective C: Elementary Mortality Survey

- Is there adequate mortality information of the AL-EEP to justify drawing conclusions regarding the health of the captive population?
- Which diseases are most prevalent in the population?
- Is disease prevalence related to time?
- Is disease prevalence related to certain zoos?
- Is disease prevalence related to the age of the individual at death?

Hypotheses:

G. Over time, there are significant differences in the frequency of different diseases. *Null = over time, there are no significant differences in the frequency of different diseases.*

H. There is a relationship between the occurrence of different diseases and time. *Null = there is no relationship between the occurrence of different diseases and time.*

I. There is a relationship between the occurrence of different diseases and zoos. *Null = there is no relationship between the occurrence of different diseases in zoos.*

J. There is a relationship between occurrence of different diseases and age at death. *Null = there is no relationship between occurrence of different diseases and age at death.*

2. Background

2.1. Conservation of the Amur Leopard

2.1.1. Status of Amur Leopard in the Wild

The IUCN Red List 2007 classifies the Amur, or Far Eastern leopard (*Panthera pardus orientalis*) as critically endangered (IUCN 2007). Recognised as a discrete subspecies (Miththapala 1996; Uphyrkina *et al.* 2001; Uphyrkina *et al.* 2002), the Amur leopard (AL) 'deserves protection as a unique genetic contribution to the species' (Miquelle *et al.* 1996). As a top carnivore, and therefore an indicator of ecosystem health and integrity (Miller *et al.* 2001), the AL is of significant regional value (Miquelle *et al.* 1996).

During the 19th century the AL occupied north-eastern China, the southern part of the Russian Far East and the Korean peninsula (see figure 2.1.a.) Today one small population of 25-44 individuals survive in southwest Primorskii Krai of the Russian Far East. A few are present in northeast China and perhaps a small number in the mountainous regions of the Democratic People's Republic of Korea (see figure 2.1.b.) (Miquelle *et al.* 1996; Aramilev *et al.* 1998; Pikunov *et al.* 1999; Uphyrkina *et al.* 2002; Kostyria *et al.* 2003; Miquelle and Murzine 2003).

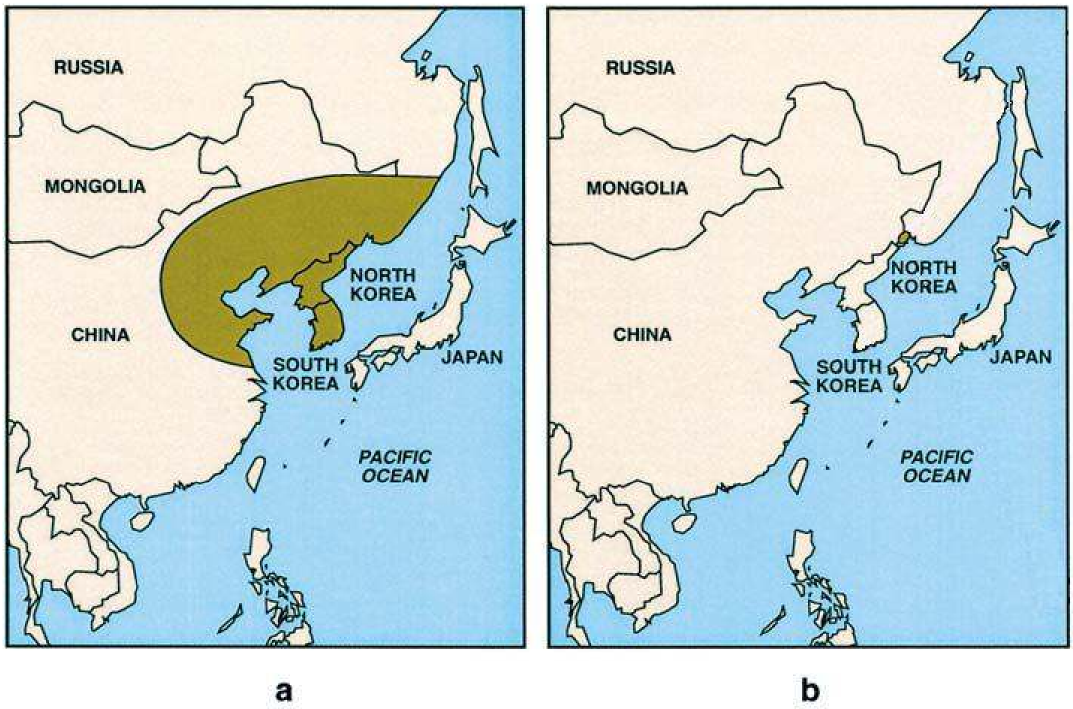

***Figure 2.1.** Geographic distribution of the Amur leopard in green: (a) 19th century, (b) present. Adapted from Uphyrkina* et al. *2002.*

Habitat destruction, elimination of prey base and direct hunting for body parts to supply the Asian traditional medicine market are some of the main reasons for reduction of the subspecies to one small fragment during the 19th century (Miquelle *et al.* 1996; Uphyrkina *et al.* 2002; Kostyria *et al.* 2003; Miquelle and Murzine 2003). Between 1972 and 2007 censuses have shown that the population size has remained stable (Pikunov *et al.* 2001; Uphyrkina *et al.* 2002; Miquelle and Murzine 2003; ALTA 2008). However, the existence of this population is fragile; loss of a few individuals due to events such as natural catastrophe, disease or inbreeding depression[2] could result in immediate extinction of the AL in the wild (Miquelle *et al.* 1996).

2.1.2. Proposed Reintroduction of the Amur Leopard

In November 1996 a group of international cat specialists met in Vladivostock, Russia, to discuss a conservation strategy to minimise the threat of immediate extinction of the AL (Shoemaker *et al.* 1996). From this meeting it was recognised that the top priority for the conservation of the Amur leopard in the wild was to continue to secure the existing population for the long-term. The value of AL reintroduction to an area within recent historical range to create a second population was also discussed. Although of a lesser priority than protecting the existing population, reintroduction could provide a safety margin should one or a combination of threats decimate the remaining population (Miquelle *et al.* 1996). These experts concluded that reintroduction is desirable, a view also supported by the IUCN/SSC (International Union for the Conservation of Nature/Species Survival Commission) Cat Specialist Group (Christie in press). The goal of the reintroduction is establishment of a second population separate from the existing leopards, with potential for exchange of individuals between the two populations in the future.

2.1.3. Sourcing Stock for Release

It is widely acknowledged that wild-caught, translocated individuals are preferred over captive bred stock for reintroduction (Jule *et al.* 2008). However, the existing population is too small to withstand removal of individuals to create this second population (Miquelle *et al.* 1996). In 2001 it was agreed that the zoo population of AL would be the source of all released cats (S. Christie pers. comm.). An additional benefit of using captive stock is the threat of inbreeding depression is reduced, as there is higher genetic diversity in the zoo population than in the wild population (Uphyrkina *et al.* 2002).

[2] *The decreased vigour in terms of growth, survival or fecundity that follows one or more generations of inbreeding. ALLABY, M. (1999)* Dictionary of Zoology, *Oxford New York, Oxford University Press.*

2.1.4. The European Captive Population of Amur Leopard (AL-EEP)

Amur leopards have been maintained in captivity since at least 1961, when a male was taken into Prague Zoo from Primorski Kraii, Russia (Miquelle *et al.* 1996). At least 32 leopards have been removed from the wild to captivity although many never reproduced and the present population is based on 9 founders (Christie in press). In 1974 IUCN and IUDZG (International Union of Directors of Zoological Gardens) approved an international studbook of AL held in captivity (Miquelle *et al.* 1996). The purpose of a studbook is to record the arrival of leopards from the wild, maintain a record of the movement of individuals between zoos and to record births and deaths.

Within European zoos there are organised captive breeding programmes for endangered species (European Endangered species Programme – EEP). The purpose of an EEP is:

- to collect information on the status of all specimens;
- to produce a studbook;
- to carry out demographic and genetic analyses;
- to produce and implement a plan for the management of a healthy population over the long term (EAZA 2008).

The Amur leopard EEP (AL-EEP) is currently co-coordinated by Sarah Christie (S.C.) of the Zoological Society of London and Tanya Arzhanova (T.A.) of Moscow Zoo. As the EEP is the main source of cats for the reintroduction programme, S.C. and T.A. have an additional responsibility to ensure sufficient stock is available. A wide variety of zoos, from Dublin to Vladivostock (Russian Far East), has held or do hold AL-EEP stock (see appendix x.x for a full list of zoos and countries). In collaboration with the AL-EEP, a North American breeding programme of AL (PMP – Population Management Programme), is currently managed at Minnesota Zoo by Diana Weinhardt.

2.2. Veterinary Considerations of Reintroduction Programmes

2.2.1. Disease

As defined by the World Health Organisation, health is 'a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity' (WHO 2006). Prevalence of disease is one way of assessing the health of a population. Disease is 'any impairment that interferes with or modifies the performance of normal functions', such as:

- responses to environmental factors such as nutrition, toxicants and climate;
- infectious agents;

- inherent or congenital defects; or a combination of these factors (Wobeser 1981)[3].

Within conservation biology, there is increasing awareness of the negative impact that infectious disease can have on wildlife populations and ecosystem health (Munson and Karesh 2002). Infectious disease is a disease caused by the entrance into the body of organisms (such as bacteria, protozoa, fungi or virus) which grow and multiply (Merriam-Webster 2008). Historically much information on the impact of disease on global biodiversity has been anecdotal (Smith *et al.* 2006). However, infectious disease has recently been implicated to a number of local and global extinctions and currently is ranked as one of the top five greatest threats to biodiversity (Wilcove *et al.* 1998). An example of the dramatic effects of disease is the rapid spread of a chitridomycete fungus. In 1998 the fungal disease was implicated in the mass mortality and localised extinction of amphibians throughout areas of Australia and Central America (Berger *et al.* 1998). However, there is recent evidence that this same disease is causing extinction of amphibian species throughout the world (Pounds *et al.* 2006; Skerratt 2007; Bielby 2008; Sodhi *et al.* 2008).

A further example of the catastrophic effect of disease is the 1993-1994 epidemic of canine distemper virus (CDV) amongst lions (*Panthera leo*) in the Serengeti National Park, Tanzania. This virus killed 35% of lions within six months and overall 85% of the population became infected (RoelkeParker 1996; Packer *et al.* 1999). Originating from CDV infected domestic dogs, surrounding the Serengeti ecosystem, the epidemic spread north into the Masai Mara, Kenya, where it infected uncounted hyenas (*Crocuta crocuta*), bat-eared foxes (*Otocyon megalotis*) and leopards (*Panthera pardus pardus*). The rapid spread and impact of CDV was due to a culmination of heightened virulence of the virus and ecological factors such as a high density of lions, their congregation in prides and interaction with other predators e.g. hyenas at kill sites (Munson 2003).

The examples given above highlight the potential far-reaching impact of disease transmission, as a result of interactions between species. Therefore, it is crucial that every reintroduction project involves veterinary expertise to assess and minimise the negative impacts of disease on the conservation efforts (Bush *et al.* 1993; Woodford and Rossiter 1994).

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[3] *Within this document "disease" will include infectious diseases (such as those caused by pathogenic bacteria, parasites and viruses) and non-infectious diseases (such as those caused by genetic abnormalities, environmental pollutants, etc).*

2.2.2. The Importance of Veterinary Input to Reintroduction Programmes

Introducing diseased individuals would compromise the ability of the founder animals to establish themselves, thus nullifying the reintroduction effort (Woodford and Rossiter 1994; Cunningham 1996). An additional concern of veterinarians is the exchange of disease between released individuals and established wildlife in the release area (Woodford 1993; Kock *et al.* 2007).

During the 1920s, 6000 Plains bison (*Bison bison bison*) were introduced from Montana into Wood Buffalo National Park (WBNP), Canada. Diseases brucellosis and tuberculosis were transmitted from the introduced animals to 1500 rare Wood bison (*Bison bison athabascae*). Attempts to eradicate and control the spread of these diseases by slaughtering Wood bison have not succeeded; brucellosis and tuberculosis in the surrounding area of WBNP continue to significantly limit the long-term recovery of the species (Woodford and Rossiter 1994; Mitchell and Gates 2002; Kock *et al.* 2007).

In some circumstances there can be a socio-economic cost to disease introduction. An example of such an impact was the introduction of European cattle into Africa, sometime during the 19th century. Unwittingly these cattle were carriers of diseases rinderpest and bovine tuberculosis. Rinderpest resulted in sporadic outbreaks in wildlife and livestock until being eradicated in the 21st century. Bovine tuberculosis spread amongst wildlife and livestock throughout southern and eastern Africa and continues to be a threat. The significant loss of cattle as a source of food has been associated with an increase in human poverty (Kock *et al.* 2007).

The examples above demonstrate the impact that disease can have on individuals and whole populations. Veterinarians have to take into account the possible effect of disease when considering reintroduction.

2.2.3. Veterinary Considerations of the Amur Leopard Reintroduction Programme

Veterinary investigation has identified the species for which exchange of disease could occur during the AL:

- between reintroduced AL and potential prey base including roe deer (*Capreolus capreolus*), sika deer (*Cervus Nippon*), domestic sika deer, wild boar (*Sus scrofa*), racoon dog (*Nyctereutes procyonides*), red fox (*Vulpes vulpes*), badger (*Meles meles*), Manchurian hare (*Caprolagus brachyurus*), feral and pet dogs and cats;
- between reintroduced AL and other felid species present in the release zone, such as the Amur tiger (*Panthera tigris altiaca*) and Far Eastern wildcat (*Felis eupilura*).

However, as leopard and tiger prey are different, there is insufficient evidence to conclude an absence of disease that could affect leopards (pers. comm. J. L.). Therefore it is essential that the AL reintroduction programme incorporates a disease management strategy.

2.3. Disease Management Strategy

It is acknowledged that reintroduction programmes will never be entirely risk free (Bush *et al.* 1993; Munson and Cook 1993). However, the risks of disease can be minimised with a disease management strategy that identifies, monitors and mitigates significant threats. A disease management strategy is comprised of various components (figure 2.2.). A theoretical disease risk assessment identifies the most significant diseases that may impact on the health of released stock and existing species in the release zone. A health assessment of wildlife and domestic species will provide baseline data of the prevalence of disease in wild populations. A comprehensive health assessment of the captive population aims to minimise transmission of disease to the wild species and assists the selection process of candidates for release. Health assessments comprise of screening results, collation and analysis of clinical (observations and veterinary treatment) and morbidity and mortality information. Screening for disease requires samples such as blood, faeces and semen. Morbidity information describes the incidence of disease in a population and mortality information details the number of deaths in a given period (Grandison 1995). Responses to the management activities are monitored and evaluated; the disease management strategy is a process under continuous review and development.

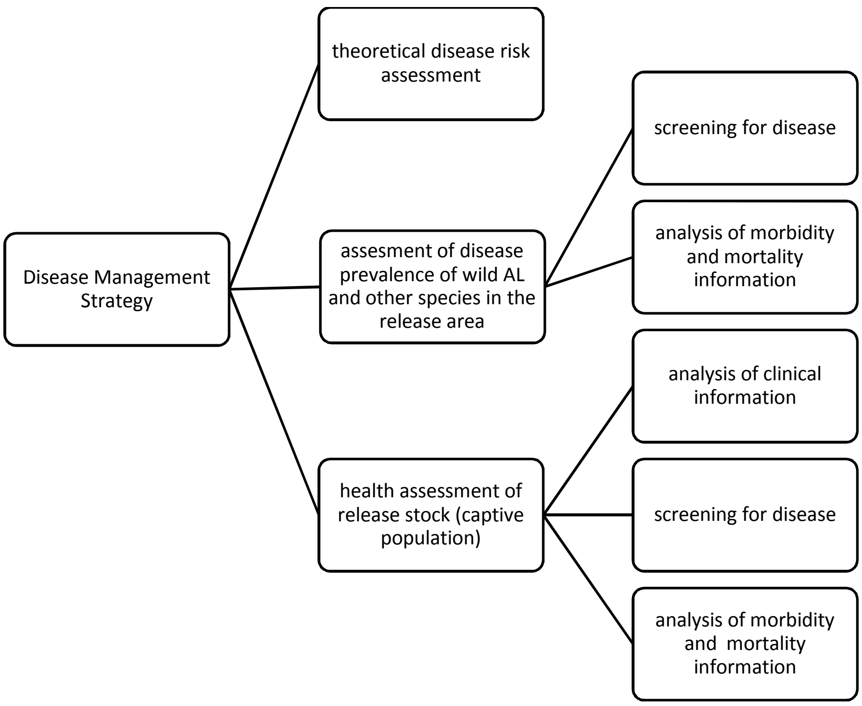

Figure 2.2. *Components of a disease management strategy (pers. obs.).*

From here on the only component of the disease management strategy that will be discussed is analysis morbidity and mortality information of release stock.

2.4. Analysis of Morbidity and Mortality Information

2.4.1. Necropsy Reports, Morbidity and Mortality Surveys

Morbidity and mortality information can inform a veterinarian of the health status of a population (Munson 1991). Morbidity and mortality information is provided by a necropsy examination 'of the internal structures of the body performed after death' which determines the cause of death and evaluates the presence of disease (West 1975).

Morbidity and mortality surveys can take the form of a retrospective analysis of all necropsy reports, for this reason necropsy reports are a particularly valuable component of any health assessment (Munson 1991). It should also be acknowledged that irrespective of the involvement of a captive population to a reintroduction programme, reasons to conduct morbidity and mortality surveys include:

- reviewing husbandry and veterinary activities in order that a healthy population is managed for the long term;
- comparing the health of different captive populations and wild populations of the same species;
- comparing the health of different captive populations of closely related species.

The following two case studies, although not specifically for captive populations involved in reintroduction programmes, provide examples of the value and use of disease assessments from captive populations.

Case Study 1: Disease Assessment of North American Captive Cheetah Population (Munson 1993)

The North American captive cheetah (*Acinonyx jubatus*) has the most comprehensive disease survey of any captive management programme (Munson 1991). Captive cheetahs have experienced a higher mortality and infertility rate, when compared with free-ranging cheetahs and other captive big cats.

Despite a small sample size of only 40 individuals over a three year period, the study increased the knowledge of disease in the cheetah population. The results of this survey identified diseases that compromised health of captive cheetahs. However, whilst this study recognised areas requiring further research, this study falls short in providing immediate practical recommendations to improve the veterinary care or captive husbandry.

The above survey and a disease survey of captive cheetahs in South Africa (Munson *et al.* 1999) were applied to an investigation of the patterns of disease between captive and free-ranging cheetahs (Munson *et al.* 2005). Despite genetic impoverishment, the health of the free-ranging population was significantly improved from that of both captive populations. This demonstrates that the response of cheetahs to the local environment is of greater importance to health than genetic factors.

Case Study 2: Morbidity and Mortality of Captive Jaguars in North America (Hope and Deem 2006)

Hope and Deem present the results of a retrospective study of the most common causes of morbidity and mortality of captive jaguar (*Panthera onca*) between 1982 and 2002. North American zoos that housed jaguar during the study period were solicited for medical records. The survey identified a high prevalence of gastrointestinal disease across all age groups. In response to this finding a protocol and recommendation for routine faecal examinations and treatment has been developed and promoted. The survey also associated a high prevalence of integument diseases to trauma from cage mates or self-mutilation, requiring a different management strategy for captive jaguar. The authors summarise that the survey provided a 'valuable overview of captive jaguar morbidity and mortality'.

These survey results enabled veterinarians and captive population managers to identify where further research was needed and produce recommendations to improve veterinary care and captive husbandry.

2.4.2. Disease Survey of AL-EEP

To date no morbidity or mortality surveys of the AL-EEP have been conducted. To implement a suitable programme, veterinarian Dr. John Lewis (J.L.) and veterinary pathologist Dr. Mark Stidworthy (M.S.) were appointed by the AL-EEP; they will be responsible for health assessment and disease management of the AL-EEP. J.L. is also responsible for the veterinary aspects of the reintroduction, including development of a disease management strategy, health assessment of release stock and selection of candidates for direct involvement in the reintroduction programme.

Source information for morbidity and mortality surveys will come from zoos. Therefore, all zoos have a responsibility to collect and provide veterinary information to enable an accurate assessment of the health of a population. In recognition of the need for zoos to submit this information, specialist global international database software has been developed.

2.5. The Use of Databases in Providing Veterinary Information

2.5.1. International Species Information Systems (ISIS)

The International Species Information System (formerly the International Species Inventory System until 1989) was established in 1973 as part of the Animal Records section at Minnesota Zoological Garden, U.S.A. ISIS was initially established as a system for the collection of specimen records in zoological institutions worldwide. Upon becoming a member to ISIS, zoos were required to submit paper copies of animal records (such as birth, death, movement and veterinary information) to ISIS headquarters where they were entered onto a pooled database (Flesness 2003).

With the advent of the personal computer during the 1980s zoos experimented with animal-record software. Recognising the potential for such data to contribute to a higher quality pooled ISIS database, in 1985 ISIS completed the development of software 'Animal Record Keeping System' (ARKS). Instead of using paper copies, member institutions were sent electronic copies of ARKS and asked to input data directly into the database and send it back to ISIS. Uptake of ARKS surpassed expectations and encouraged the development and improvement of ARKS to ARKS version 2 in 1987 (Flesness 2003). There has been continued financial support for the development of ARKS to the current version 5.

To facilitate management of captive populations, ISIS released SPecies Animal Record Keeping System (SPARKS) software in 1987. Those managing captive breeding programmes were responsible for collecting from zoos and inputting data of every individual in the population into the database. SPARKS became the world studbook standard as an inventory and source of genealogical data of individual animals in a captive population. Compatibility with and distribution of genetic and demographic analytical software with SPARKS have contributed to the success of this database product for use in captive population management (Flesness 2003).

At a meeting of American Association of Zoo Veterinarians (AAZA) in 1986, ISIS was asked to develop software for veterinary records in order to pool and standardise veterinary data. Medical Animal Record Keeping System (MedARKS) was developed. A benefit to using MedARKS for entry of veterinary information is that the database structure and options available during data entry result in a description of veterinary data in a standardised format. Veterinary departments of zoological collections are responsible for entering data into the database. Pooled veterinary data is updated annually enabling veterinary projects to generate normal values for haematology and serum chemistry for over 800 wildlife species managed in zoos (Flesness 2003).

Although current ISIS databases such as ARKS, SPARKS and MedARKS are compatible with each other (for example each specimen of a species has one unique identification number used across all ISIS software), they are distinct entities.

2.5.2. Limitations of MedARKS and Other ISIS Databases

In principle, adequate morbidity and mortality information for a health assessment of a captive population can be extracted from MedARKS. However, there are several limitations to MedARKS that prevent this data being available. Potential value of the MedARKS as a source of veterinary data for the purpose of research and analysis of husbandry practice can only reached if all data is recorded. It is the responsibility of the zoo to input individual records into ISIS databases. This requires that every zoo:

- is a member of ISIS;
- subscribes to the databases in question;
- collects the data;
- has the resources to input data;
- has the expertise to input accurate detailed veterinary records for MedARKS.

Flesness state that 'MedARKS was being used at approximately two hundred ISIS member institutions' in 2000 (Flesness 2003). With 595 ISIS members at the end of 2001 (Flesness 2003), there is a MedARKS uptake rate of only one third of ISIS members. Furthermore this does not take into account the number of zoos that are not a member of ISIS. For example, of approximately 40-50 zoos in the UK only three (Bristol Zoological Gardens, Chester Zoo and Durrell) are subscribers to MedARKS (pers. comm. M. Kelly). However, although these zoos are subscribers to MedARKS they are not necessarily actively recording data into MedARKS.

Lack of data collection in the first instance may explain an absence of veterinary information on ISIS databases. For zoos that do collect data there need to be staff with sufficient time for data entry into the database. There are practical issues with MedARKS that hinders data entry and use (pers. obs.):

- the database runs only on MS DOS mode; when in use, unless all other programmes running the on the computer are shut-down the software frequently freezes;
- the database is not user-friendly, it takes time to learn how to navigate the software;
- extraction of data from MedARKS for analysis is challenging.

Various unsatisfactory elements as outlined above means that there is no guarantee of the quality of the data entered into MedARKS. Flesness neglects to discuss the quality of the existing '3.2 million standardised veterinary records' (Flesness 2003). It is possible that entire veterinary records for an individual can consist only of birth and death dates, and thus will be uninformative when conducting research.

As an example of a limitation of the ISIS database system, an assessment of the difference in ISIS data and ten studbooks (see section 2.1.4. for a definition) was completed by Earnhardt and Willis (1995). The authors describe that 19.2 ±2.2% of information in ISIS databases differed from studbooks. The authors conclude that the ISIS database is not suitable for population management until there is 'an increased commitment to data quality by record keepers, zoological institutions, and ISIS' (Earnhardt and Willis 1995).

For scientific analyses and research to inform captive population managers there needs to be 100% submission of comprehensive accurate and detailed data entered into the database network. Supervision of data entry would assist this process, but ultimately zoos need to take on the responsibility to collect information for every specimen held, and then careful and accurate input data into an appropriate database that is widely accessible to researchers.

2.5.3. CAPTIVE Database (Bailey et al. 2003)

Bailey *et al. (2003)* describe the development of CAPTIVE - a bespoke database constructed in Microsoft Access version 2.0. CAPTIVE was designed for the storage and manipulation of biomedical and aviculture data for bustard captive breeding programmes at the National Avian Research Breeding Center (NARBC), Abu Dhabi (Bailey *et al.* 2003). Rather than using existing software such an ISIS database, which provide little opportunity for customisation, the authors discuss their preference to create a database to meet their specific needs. During development the main criteria of CAPTIVE was to ensure:

- user-friendliness;
- consistency in data entry;
- ease of data retrieval;
- the ability to analyse large amounts of data rapidly.

NARBC animal keeping staff initially record relevant information on paper; they then submit a copy to the animal records personnel for data entry into CAPTIVE. The main function of the database is to provide veterinary reports and build up accurate veterinary profiles. Bailey *et al.* conclude that the database met requirements of the NARBC in reviewing the efficacy of veterinary procedures, and informing captive management decisions of the species concerned. The main disadvantage to developing a bespoke database is a change of personnel. However, Bailey comments that this situation can be prevented with good documentation to support new user to CAPTIVE.

Despite attempts by the thesis author to make contact with the Bailey and after internet searches for CAPTIVE, it seems that no attempt has been made to make the database widely available to other captive population managers.

2.5.4. Zoological Information Management System (ZIMS)

Zoological Information Management System (ZIMS) is a new animal record keeping system currently under development. The mission of the ZIMS project is 'to develop and maintain a new and improved information system that supports a wide range of animal management and conservation activities in zoos and aquariums' (ISIS 2008). By providing a means to share information of all species held in zoos across the world, it is hoped that ZIMS will facilitate research of captive population. In contrast to the current ISIS databases, ZIMS will be integrated to include modules for animal inventory, veterinary care, nutrition, husbandry, environmental monitoring, collection planning and research. The new software is being designed for the importation of data from existing ISIS software whilst also allowing for future expansion (ISIS 2008). Unfortunately, although originally due for release mid-2006, ZIMS is currently running significantly behind schedule and is not expected to be available in the very near future.

2.5.5. Veterinary Records of the AL-EEP

A SPARKS database for the AL-EEP is maintained and updated by the EEP co-coordinators S.C. and T.A.. However, the data for A.L. in ISIS databases is incomplete and unsuitable for the purpose of extracting veterinary records, morbidity and mortality data for a health assessment of the population (pers. comm. J. L.). Once ZIMS has been developed it should provide the vehicle to extracted required data. However, the success of ZIMS in becoming a unified global database will largely depend upon uptake of the software by institutions, the extent of data collection and input each zoo contributes to the system. More importantly, there is an urgent need for a health assessment of the EEP population of Amur leopard for the imminent reintroduction programme, there is no time to wait for ZIMS.

For a comprehensive morbidity and mortality survey there needs to be the collation of all necropsy results from every specimen that has ever been in the AL-EEP. Once this information is gathered it needs to be organised and stored in a manner that is user-friendly, accessible and enables analysis of the data to assess the overall health of the AL-EEP. The importance of this data cannot be overemphasised; it is a crucial component of the disease management strategy for the reintroduction programme for the conservation of the subspecies in the wild.

3. Methods

3.1. Data Collection

Since 2002 J.L. has solicited all AL-EEP zoos requesting zoos to confirm whether a necropsy examination had been conducted, and to provide any reports. Contact with zoos has been made via email, dissemination of AL-EEP annual reports requesting information, presentations given at European Association of Zoos and Aquaria conferences and face-to-face talks with zoo personnel. J.L. has also extracted as much data as possible from existing ISIS databases. Mortality information of the AL-EEP occurs in the following formats:

- *necropsy report:* a report from a zoo to confirm a necropsy examination occurred and to present a conclusion on cause of death and any other morbidity observations (see appendix 1 for an example of a comprehensive necropsy report);
- *ISIS database software comment:* there is some indication that a necropsy examination may have taken place via a comment on either ARKS, MedARKS or SPARKS software (see appendix 2 for two examples) and data has been extracted by J.L.;
- *note:* pathology information communicated directly to J.L., that is not a necropsy report (see appendix 3 for two examples);
- *none:* the zoo has confirmed that there is no pathology information available for the individual in question;
- *outstanding:* awaiting a response from the zoo for mortality data.

Where necessary J.L. obtained translation of information into English where necessary. Prior to the start of this thesis, all veterinary information, such as pathology, clinical, screening and sample storage information were entered into Microsoft Access 2007 in spreadsheet format by J.L.. The spreadsheet was made available to the author for the purposes of the thesis.

3.2. Database Development

3.2.1. Database Design

With guidance from S.C. a database was designed in Microsoft Access 2007 for sake of simplicity and ease of availability. Important criteria for the database are to be 'user-friendly' and supportable by someone else upon completion of the thesis. The database was designed to store mortality information required for thesis objectives, in addition to all other veterinary data such as clinical, screening and sample data. However, only the design and data entry of aspects of the database relating to mortality data will be discussed further.

3.2.2. Data Input

Mortality data vary widely as a result of originating from various formats (as in section 3.1.) and being collected and written by different practitioners from across Europe and Russia. For the categorisation of mortality data in, there are three levels of organisation in the database:

1. Type of mortality information:
   - *cause of death (CoD):* the principal cause of death of the individual, can be a disease (e.g. pneumonia) or the consequence of an action (e.g. euthanasia);
   - *related pathology:* factors that contributed to, but are not the principal cause of death;
   - *incidental pathology:* do not contribute to the cause of death, but are diseases or unusual observations identified during a necropsy examination.

2. CoD, related and incidental pathology data are described in three categories:
   - *disease process (DP):* description of the disease condition e.g. haemorrhage;
   - *topography:* the description of an anatomical region or part e.g. heart;
   - *aetiology:* the cause or origin of disease e.g. feline immunodeficiency virus.

For each of the above categories a list of all possible definitions and the standard terminology to use has been taken from MedARKS. It is required that for each animal that has died in the EEP there be a DP, topography and aetiology for cause of death entered into the database. In each category 'unknown' is an option.

3. Confidence of the CoD being accurate is dependent on the amount of available evidence to support the conclusions made in the necropsy report/note/comment. The mortality information should present all evidence indicative of the cause of death. During review of pathology information for each cat, by M.S. and J.L. to graded how confident they were of the CoD described in the necropsy report/note/comment to one of the following confidence categories:
   - *conclusive:* given the evidence in the report there is little doubt about the conclusions drawn;
   - *presumptive:* the evidence gives a reasonable basis for the conclusions drawn;
   - *suggestive:* based on the evidence there is a hint of the conclusions drawn;
   - *unestablished:* there is not enough evidence on which to draw a conclusion.

During data entry all mortality information was carefully reviewed to ensure that similar terminology was used and to smooth over medical differences of opinion. The veterinary pathologist to the AL-EEP, Dr. Mark Stidworthy (M.S.) discussed every piece of mortality information with J.L. to decide how to categorise the information. Data for analysis was then extracted from the database.

Figure 3.4. summarises in chronological order the process of mortality information from various sources into the database, and parts of the information extracted for analysis in this thesis.

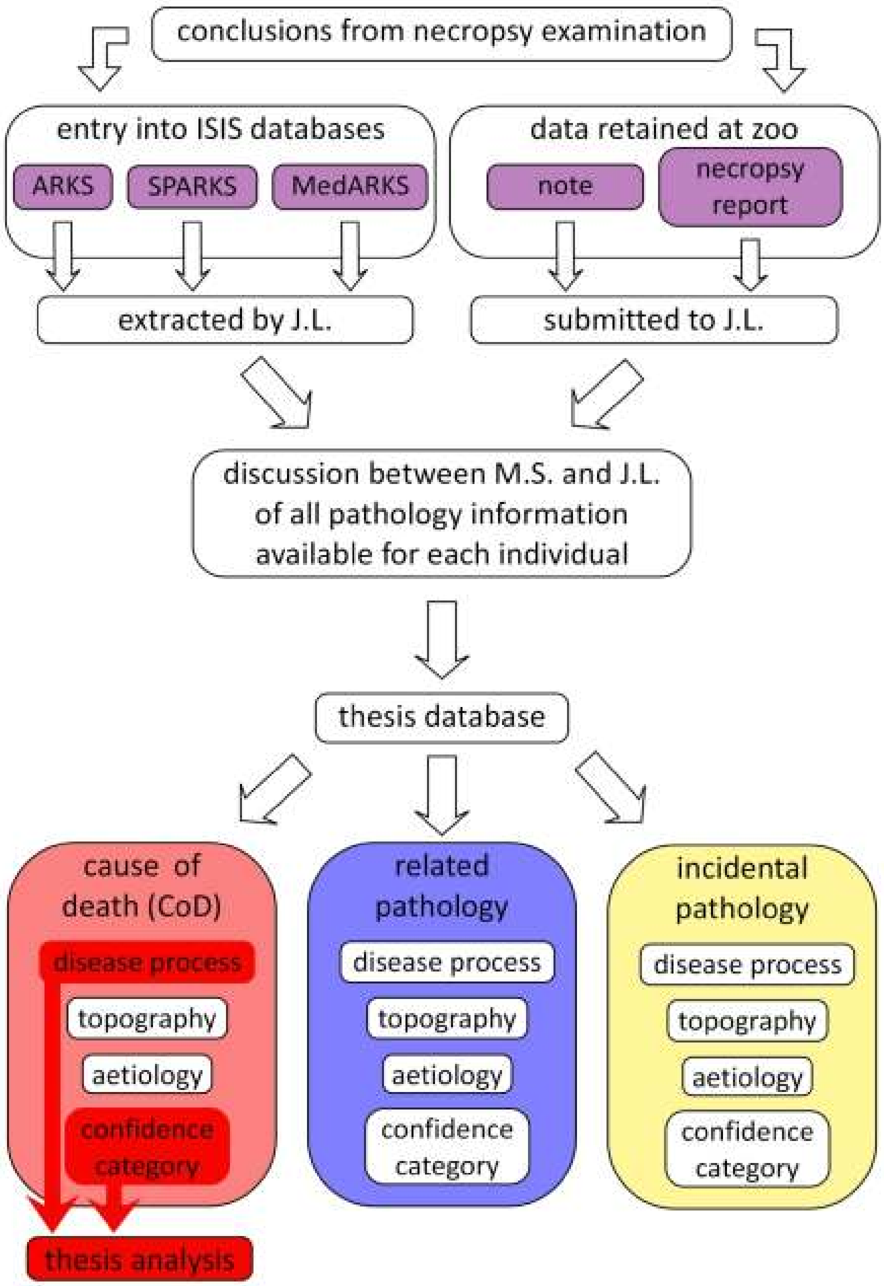

Figure 3.4. *Summary of data origin, collection and database design. Purple boxes are types of pathology information, red boxes indicate cause of death data and two categories used during analysis.*

3.3. Data Used for Analyses

Data was extracted from the database into statistical computing programme 'R' version 2.7.1. (RDevelopmentCoreTeam 2008). Graphs were constructed in either Microsoft Office Excel 2007 or in 'R'.

3.3.1. Necropsy Examinations and Recording of Information

Some cats were born in the EEP and then moved to a non-EEP zoo (for example to the USA) and then a necropsy report has been submitted to Lewis. However, this thesis is concerned with the behaviour of EEP zoos and therefore only those cats that have died at EEP zoos from the beginning of the EEP in 1969 until 18th July 2008.

For each individual mortality data can exist in more than one format (see section 3.1.), the highest quality data type was used to categorise what format the mortality information was available.

3.3.2. Extent of Necropsy Report Writing (Hypotheses A, B, and D)

To account for variation in sample sizes over time (the population size has considerably increased from nine founding animals) the overall frequencies of necropsy examination performed and the number of necropsy reports written were used to calculate proportions for use during analysis.

3.3.3. Quality of Necropsy Reports (Hypotheses D, E, and F)

The quality of a necropsy reports can be gauged by the amount of evidence produced to support the conclusions drawn (see section 3.2.2.). Throughout this thesis a good quality necropsy report are those that have a confidence category as 'conclusive' for the CoD.

In acknowledgement that for necropsy reports that required translating, the quality of the translation is just as important as the quality of the report; veterinarians have been approached to translate reports. Three necropsy reports were not included in this analysis as they are awaiting translation and thus it would be unfair to comment.

3.3.4. Elementary Mortality Survey (Hypotheses G, H, I and J)

Pathology data from ISIS databases and notes from zoos are usually brief (see appendices 2 and 3) with little evidence to support conclusions made. Therefore, necropsy reports are the only source of data used in a mortality survey of the population. As an indicator of the type of diseases existing in the population – the frequencies of different disease processes (see section 3.2.2) were analysed. There are 209 possible disease processes listed in the database (see database structure in appendix 5). 'Unknown' is applied when the cause of death could not, and will never be established, and 'not applicable' for instances when the necropsy report is awaiting translation and more information will follow. Only frequently occurring diseases were used for analyses in time, between zoos and age of individual at death.

3.3.5. Time Analyses (Hypotheses B, E and H)

To take into account the variable size of the population since 1969, the proportion of necropsy reports for all dead cats had to be used in time analyses rather than overall frequencies. The first Amur leopard to die in the EEP was 11th July 1969, and the cut off date was 18th July 2008, eight categories were chosen. One of six years between 1969 and 1974 inclusive, six of five years between 1970 and 2004, and one of three years between 2005 and 2008.

3.3.6. EEP Zoos Analyses (Hypotheses C, F and I)

All EEP zoos that have ever had Amur leopard deaths were included in the analysis. Zoo locations range from Ireland, West and East Europe and across Russia, see appendix 4 for a full list of zoo locations.

3.3.7. Time and Zoo Analyses (Hypotheses D and G)

Criteria for selection for hypothesis D are zoos that had at least 10 deaths spanning over a minimum of 20 years. Zoos selected for analysis in hypothesis G had written at least four necropsy reports spanning over a period of at least ten years.

3.4. Statistical Analyses

All statistical analyses were conducted in R version 2.7.1. (RDevelopmentCoreTeam 2008). For proportion data an ANOVA using chi-square of a generalised linear model (GLM) with binomial errors of the data and a null GLM model with no variation was used as a deletion test. An analysis of deviance with binomial errors was used for hypotheses D and G relating to both time and zoos (two categorical explanatory variables) and an ANOVA deletion test. A Fisher's exact test was used to where a chi-squared distribution could not be assumed (Crawley 2007). Test assumptions are that there are no zero frequencies in any cell and that at least 80% of all cells have a count of at least 5.

4. Results

4.1. Objective A: Extent of Necropsy Report Writing and Submission

4.1.1. Necropsy Examinations and Recording Observations

The date of the first death in the captive population was 11th July 1969. Up to 18th July 2008, a total of 249 cats have died. Of these deaths, 51% are known to have been necropsied and 10% are known to have had a necropsy examination. For the remaining 39% of deaths it is unknown whether a necropsy examination was conducted or not (table 4.1.).

Necropsy Examination Conducted	
yes	51%
no	10%
unknown	39%

Table 4.1. *Extent to which necropsy examinations were conducted, as a percentage of the number of deaths (n = 249).*

There has been a 91% response rate to solicitations by Lewis for mortality data, 92 necropsy reports (37% of all deaths) have been submitted (figure 4.1.). Comments from ARKS, MedARKS and SPARKS in addition to notes sent to Lewis account for 43% of deaths. For 12% of deaths (29 individuals) is it known that no mortality information is available. For the remaining 9% of deaths (23 individuals) mortality information remains outstanding.

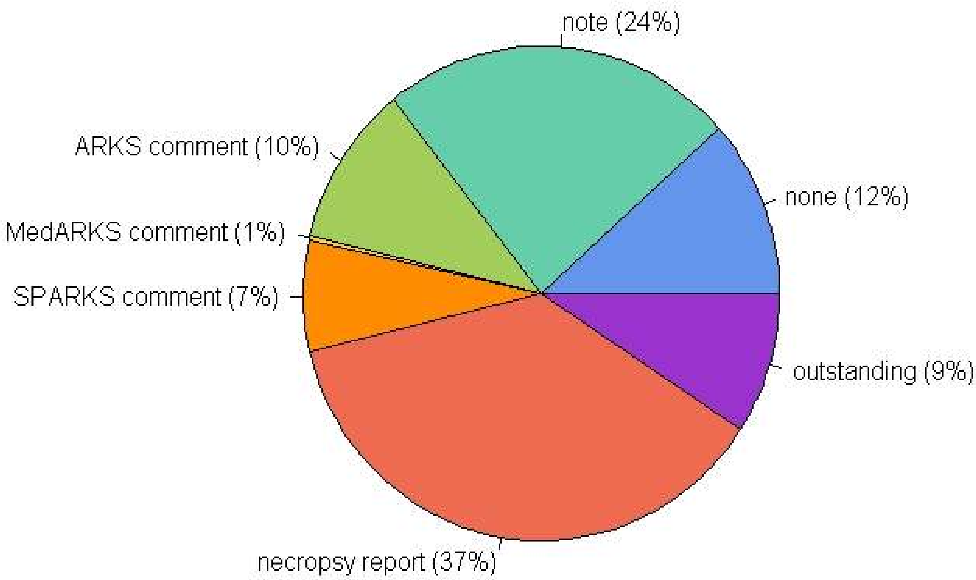

Figure 4.1. *Format of mortality information available for deaths in the AL-EEP (n= 249).*

For those deaths where little information was available, there were difficulties in establishing whether a necropsy examination was carried out or not. This is reflected in a high percentage of deaths (39%) which it was 'unknown' whether an individual had been necropsied (table 4.1.). Therefore analysis of zoos producing necropsy reports, rather than the extent of necropsy examinations was used to investigate the following hypotheses.

4.1.2. Hypothesis A: Over time there has been an increase in the proportion of deaths for which there is a necropsy report

Amongst all time categories, the proportions of deaths with a necropsy report varies between 0.30 and 0.60 (figure 4.2.), with a mean 0.44.

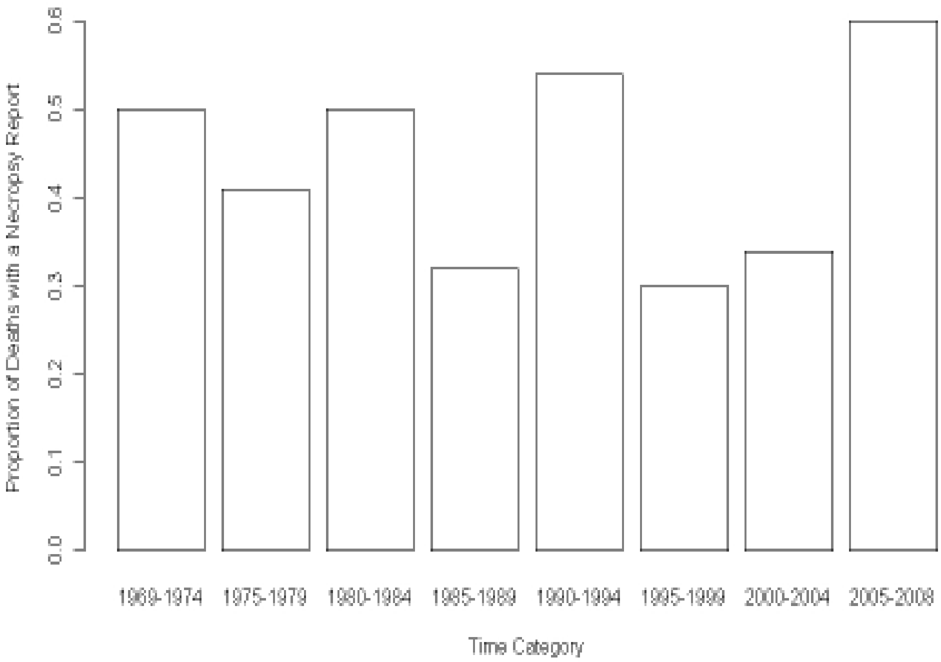

Figure 4.2. *Proportion of deaths for which a necropsy report has been written and submitted to Lewis, for each time category.*

There has been a recent increase from 0.30 and 0.34 deaths with a necropsy report between 1995-1999 and 2000-2004 respectively, to the highest proportion (0.60) during 2005-2008 (figure 4.2). However, overall time there is no significant difference in the proportion of deaths for which a necropsy report was written with time ($p>0.5$). The null hypothesis cannot be rejected, and the alternative hypothesis cannot be accepted.

4.1.3. Hypothesis B: Between zoos there is a significant difference in the proportion of deaths from which there is a necropsy report

Of 45 zoos in the Amur leopard EEP, 17 zoos (38%) have submitted at least one necropsy reports; five zoos have done so for more than 0.80 of deaths at the zoo (figure 4.3.).

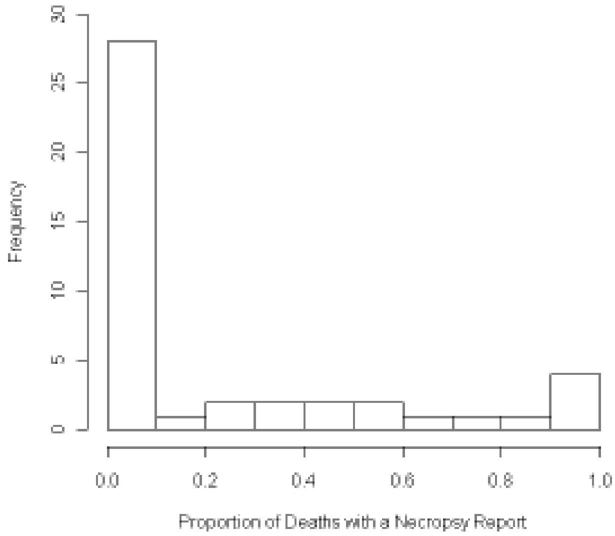

***Figure 4.3** For every zoo in the Amur leopard EEP (frequency, n = 45), the histogram shows the proportion of deaths for which there are necropsy reports.*

The mean proportion of deaths with a necropsy report (0.224) is substantially bigger than the median (0.000), demonstrating a strong negative skew (figure 4.3.). There are significant differences amongst zoos in the proportion of deaths for which there are necropsy reports ($p < 0.001$). A low proportion of necropsy reports available from zoos results in rejection of the null hypothesis of no difference in proportion of deaths with a necropsy report. The hypothesis is accepted - there is a significant difference between zoos in the proportion of deaths for which a necropsy report is written.

However little weight should be placed on figure 4.3., without taking into account the number of deaths at each zoo. A post hoc investigation analysis demonstrated a significant relationship ($p < 0.001$) between the number of deaths at a zoo and proportion of deaths for which a necropsy report is written (figure 4.4.).

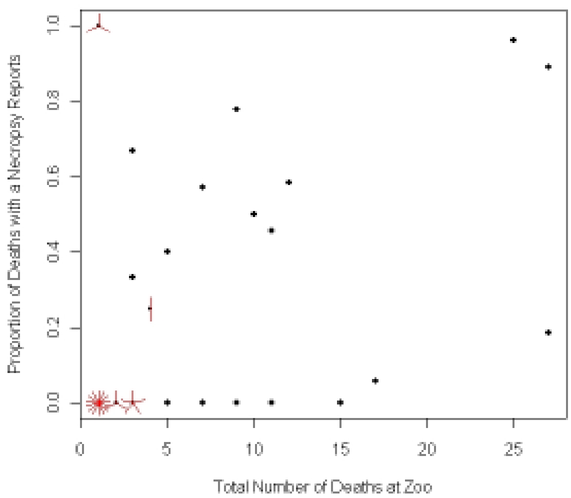

***Figure 4.4.** Each data-point represents a zoo, plotted according to the proportion of deaths for which there is a necropsy report and the number of deaths in that zoo. Red lines surrounding a data-point represent the frequency of zoos with that value.*

4.1.4. Which zoos have a poor compliance rate?

Figure 4.5. is a breakdown of the proportion of deaths in each zoo for which there have been a necropsy report, arranged by the numbers of deaths in each zoo.

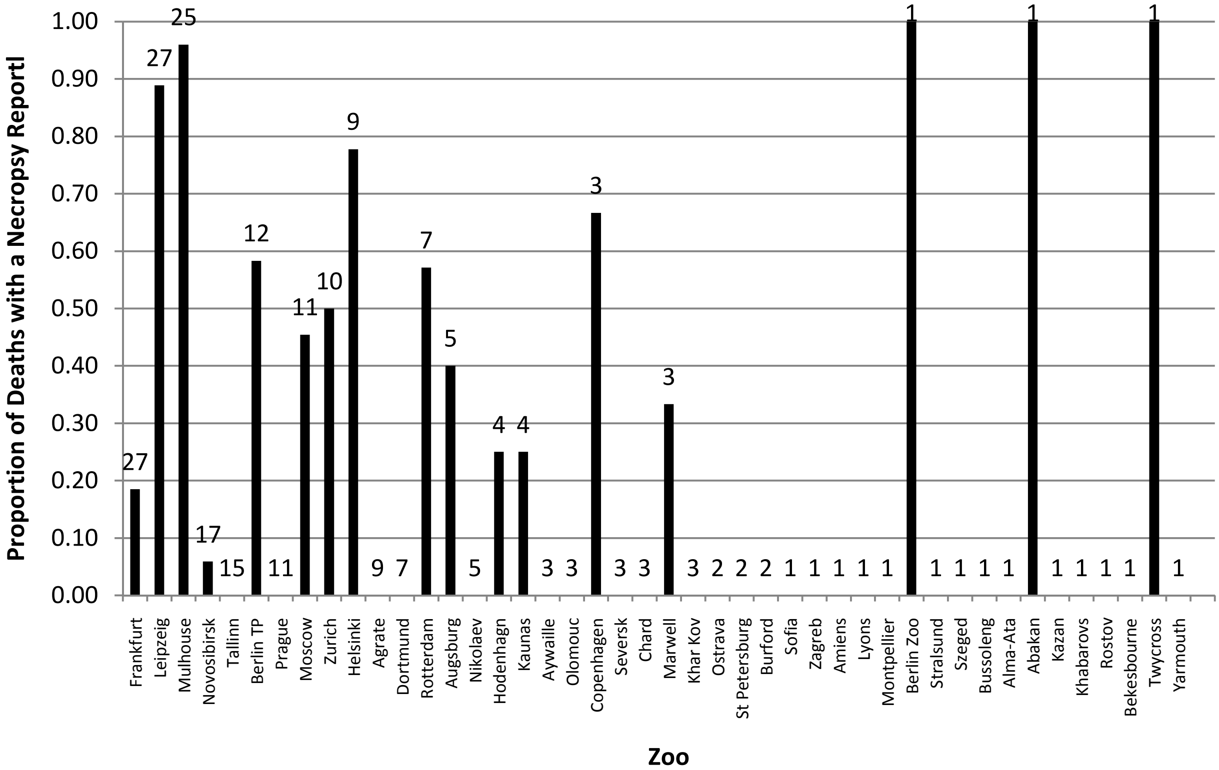

__Figure 4.5.__ Proportion of deaths for which there is a necropsy report from each zoo in the AL- EEP, arranged by the number of deaths in the zoo (value on top of the bars).

The following zoos have had more than ten deaths, but very few necropsy reports have been written:

- *Frankfurt, Germany* have seen 27 deaths, however there are only five necropsy reports, 21 ARKS comments and one note to J.L. Referring back to the database reveals that all ARKS comments were for deaths prior to 1990. There are necropsy reports for all deaths between 1992 and 1997, with exception of one note.
- *Novosibirsk, Russia* have had 17 death between 1978 and 2007. The only necropsy report available is for the most recent death. Pathology data remain outstanding from three deaths in 2005, and there is no pathology information available from a cat that died in 2003.
- *Tallin, Estonia* have seen fifteen deaths for which all pathology data are notes except for one SPARKS comment. Nine have occurred between 1972 and 1977, five between 1993 and 1998 with the most recent in 2006 for which there was a note.

- *Prague, Czech Republic* have had 11 deaths although none with a necropsy report. Since 2001 there have been five deaths, with three notes submitted to J.L. and two individuals (both in 2002) for which there is no pathology information available.

4.1.5. Hypothesis C: Over time, zoos exhibit a similar pattern in proportion of deaths for which a necropsy report is written

Eight zoos met the criteria for analysis (section 3.3.7). Figure 4.6. shows the proportion of necropsy reports written for deaths in each time category in the zoos with leopards over an extended period of time.

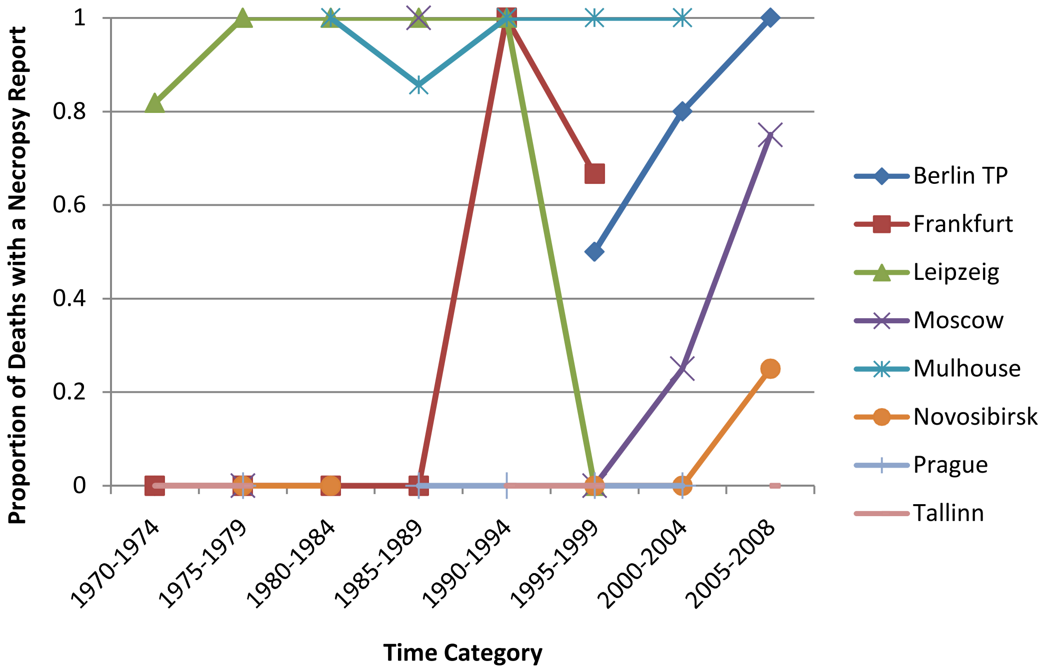

Figure 4.6. *Proportion of deaths for which there has been a necropsy report in eight zoos over eight time categories.*

A null hypothesis that zoos do not exhibit similar patterns in proportion of necropsy report writing over time cannot be rejected ($p > 0.5$ for all zoos). The hypothesis cannot be accepted.

4.2. Objective B: Quality of Necropsy Reports

4.2.1. Hypothesis D: Over time there is a significant increase in the proportion of necropsy reports with a conclusive cause of death

Of 89 necropsy reports of the Amur leopard EEP, 23 (26%) have described a conclusive diagnosis of cause of death.

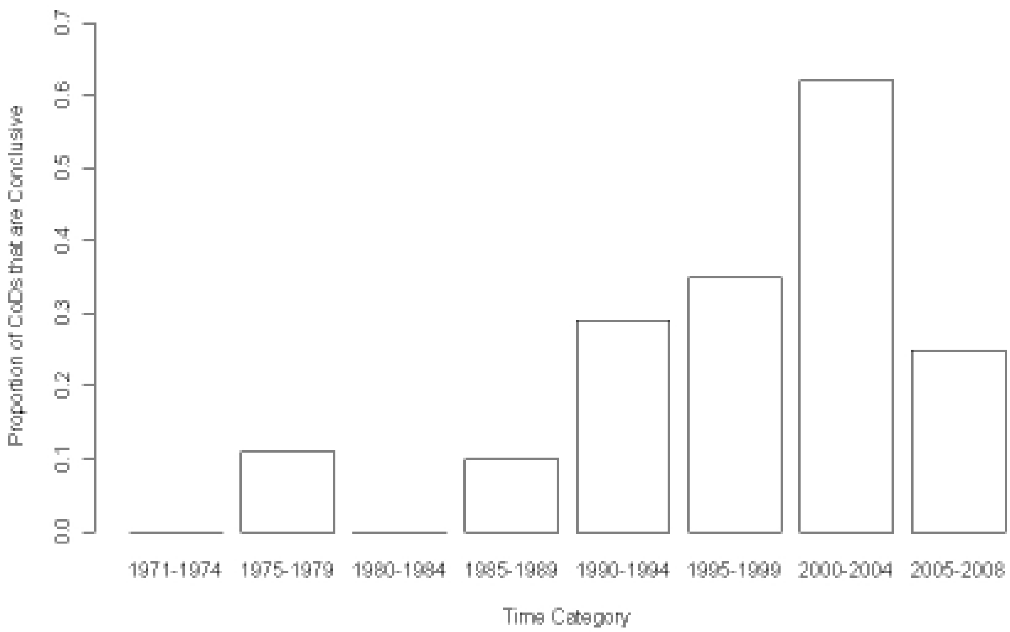

Figure 4.7. *The proportion of necropsy reports for which there was a conclusive cause of death (CoD) in each time category.*

Overall there is an increase in the proportion of CoDs with a conclusive CoD, except for a reduction between 2000-2004 and 2005-2008. Despite this, there is a significant difference between time and the proportion of deaths for which a necropsy report was written ($p < 0.01$). Using the confidence in CoD as a proxy for the quality of a necropsy report (section 3.3.3.), a null hypothesis of no significant difference is rejected, the hypothesis that the quality of necropsy report have improved can be accepted.

4.2.2. Hypothesis E: Between zoos there is a significant difference in the proportion of necropsy reports for which there are conclusive cause of deaths

Of 17 zoos in the Amur leopard EEP that have submitted necropsy reports, four zoos have described a conclusive cause of death for 100% of deaths (figure 4.8.).

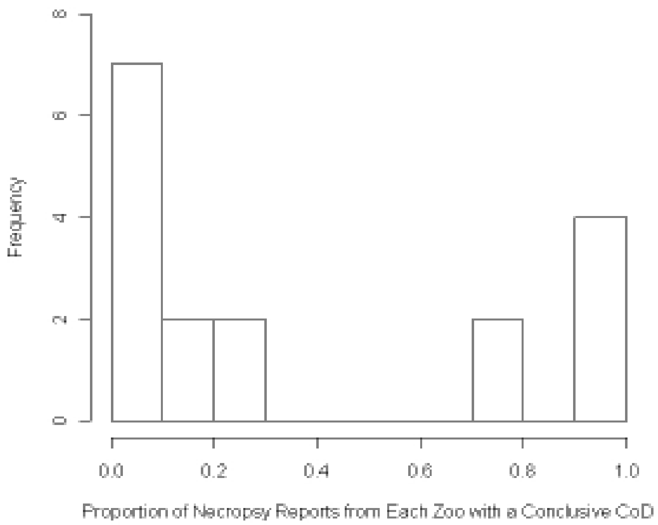

Figure 4.8. *Proportion of all necropsy reports from each zoo, for which there has been a conclusive cause of death (CoD).*

The mean proportion of cause of deaths that are conclusive (0.3788) is substantially greater than the median (0.200), supporting the negative skew toward a low proportion seen in Figure 4.8. Substantially ($p < 0.01$) more zoos provide fewer conclusive cause of death than a high proportion of necropsy reports for which there is a conclusive cause of death. The observed non-normality towards a low proportion results in rejection of the null hypothesis that there is no difference in the proportion of necropsy reports that are good quality, the hypothesis is accepted.

The strength of the observation is reduced as all four zoos that provided conclusive cause of deaths only wrote one necropsy report. A *post hoc* investigation of the number of necropsy reports written by a zoo and the proportion of conclusive cause of death data is illustrated in figure 4.9.

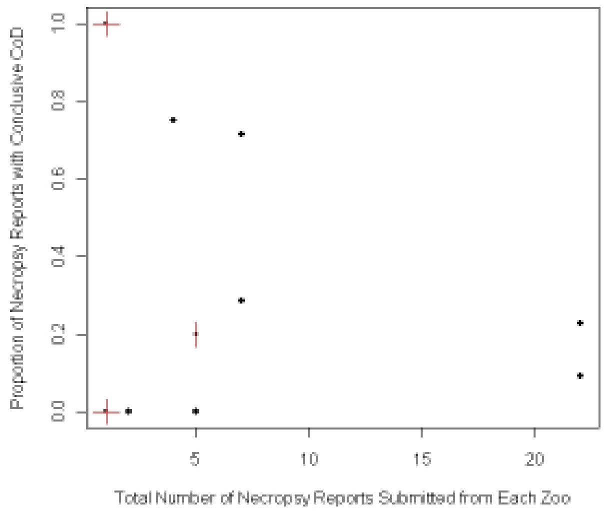

Figure 4.9 *Total number of necropsy reports submitted from each zoo and the proportion of those reports that give a conclusive diagnosis of the cause of death (CoD). Red lines surrounding a value represent the number of zoos with that identical value.*

There is a greater proportion of conclusive cause of death with increasing number of necropsy reports written by each zoo ($p < 0.05$).

4.2.3. Which zoos are writing poor quality necropsy reports?

Figure 4.10. shows the proportion of all necropsy reports written by each zoo that have a conclusive CoD. Mulhouse and Leipzeig have written many necropsy reports, although they are overall of a low quality – as gauged by the small proportion of necropsy reports with a conclusive CoD.

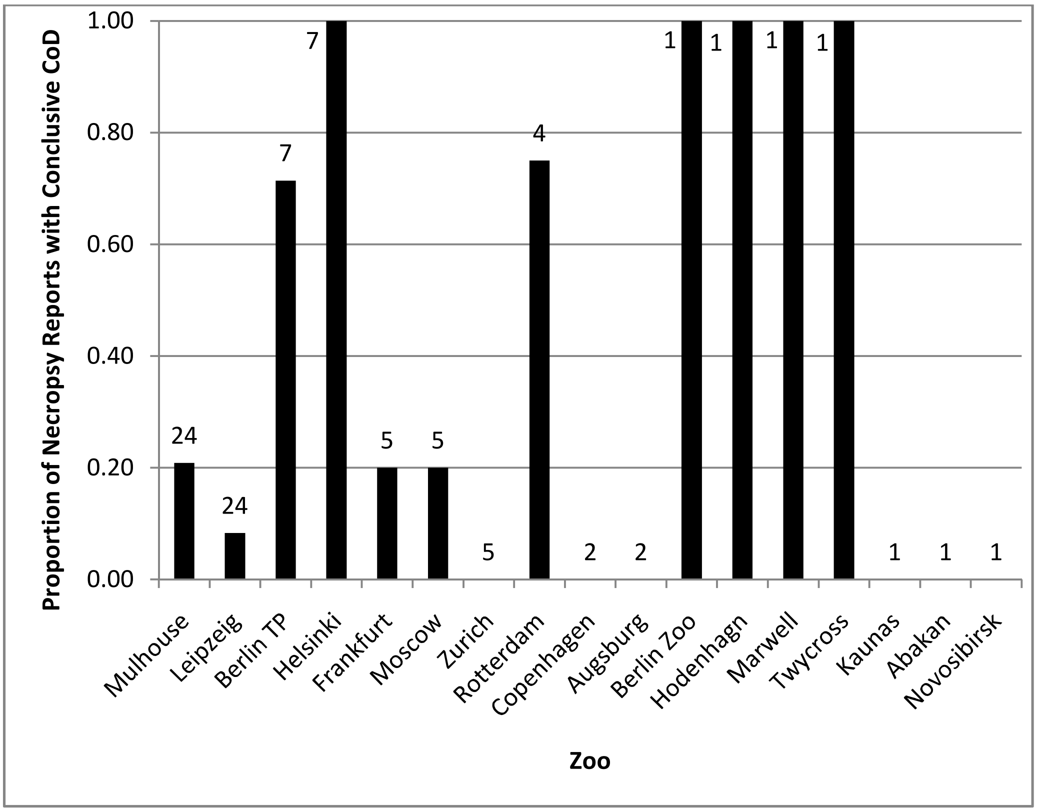

Figure 4.10. *The proportion of all necropsy reports from each zoo (frequency, n= 17) that describe a conclusive cause of death (CoD).*

4.2.4. Hypothesis F: Over time, individual zoos exhibit a similar pattern in the proportion of necropsy reports with a conclusive cause of death

Five zoos met the criteria for analysis – see section 3.3.3. Figure 4.11. illustrates the proportion of necropsy reports with a conclusive CoD in the zoos over time.

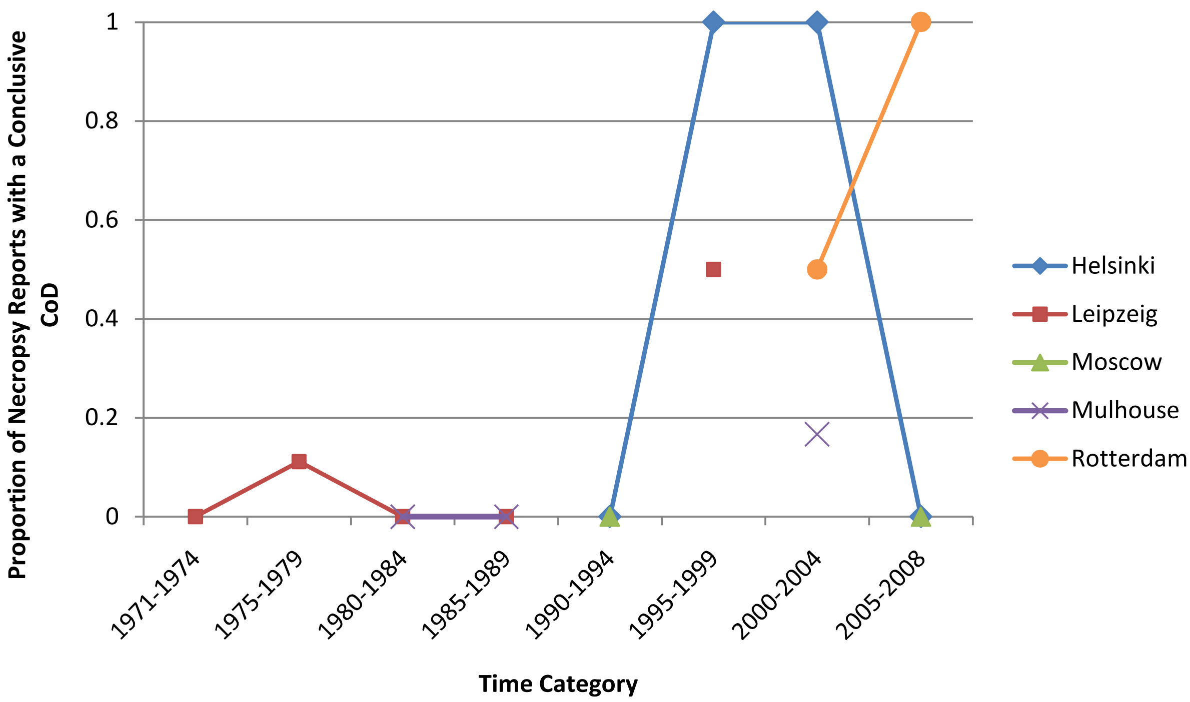

***Figure 4.11**. Proportion of necropsy reports that described a conclusive cause of death (CoD) in five zoos over eight time categories.*

Across zoos there is no significant relationship between time and the proportion of necropsy reports with a conclusive cause of death ($p > 0.05$). A null hypothesis that all zoos vary in the proportion of conclusive cause of deaths cannot be rejected, and so the hypothesis is rejected.

4.3. Objective C: Elementary Mortality Survey

4.3.1. Hypothesis G: Over time, there are significant differences in the frequency of different diseases

Of 92 necropsy reports, 29 disease processes were identified from a possible list 209 with additional 'not applicable' and 'unknown' categories (see section 4.4.1.). Figure 4.12 illustrates the frequency of those diseases that occurred and were the cause of death according to necropsy reports.

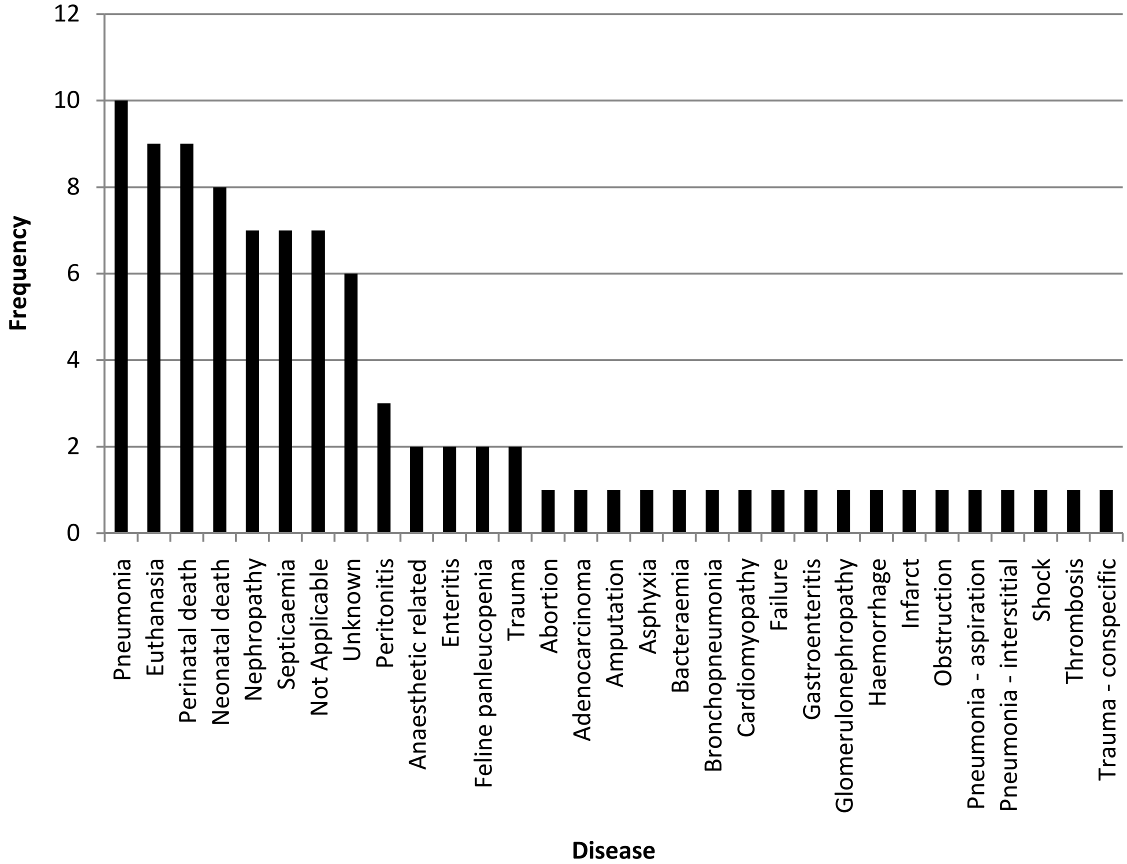

Figure 4.12 *Histogram of the frequency of all disease processes in all necropsy reports (n = 92) – distribution of disease occurrence in all necropsy reports*

There are some diseases that result in more mortality than others (figure 4.12). Those with the highest prevalence are pneumonia, euthanasia, perinatal death, neonatal death, nephropathy and septicaemia.

Taking into account the general pneumonia category along with cases of bronchopneumonia, aspiration and interstitial pneumonia, 15% of all necropsy reports attributed CoD to a form of pneumonia - inflammation of the lung (West 1975). Perinatal death (within three months before and one month after birth) occurred in 10% of all individuals with a necropsy report.

For euthanasia (10% of cats with a necropsy report) and neonatal deaths (death few months after birth) related pathology information reveals diseases that lead up to the death. Six of the nine euthanasia have the following related pathologies (therefore contributing to the CoD):

- degeneration of the eye
- cardiomyopathy
- trauma in abdomen
- auto-mutilation of skin
- encephalopathy
- pneumonia

The remaining three euthanasia cases do not describe related pathologies that lead to euthanasia.

Of eight neonatal deaths (9% of cats with a necropsy report), related pathologies are:

- maternal neglect
- 2 x starvation due to inflammation of larynx
- starvation

The remaining four neonatal deaths do not describe any related pathologies.

Nephropathy, also known as nephrosis is disease of the kidneys (West 1975) and septicaemia (circulation of bacteria in the bloodstream – West 1975) each account for 8% of all cats with a necropsy report.

There is substantial variation amongst the frequency of different diseases and a difference in the mean (2.968) and median (1.000) and a hugely significant difference ($p < 0.001$) to reject a null hypothesis. The hypothesis is accepted - there is significant difference in the frequency of disease causing death in the Amur leopard population.

4.3.2. Hypothesis H: There is a relationship between the occurrence of different diseases and time

The proportion of deaths in each time category that was attributed to the six most prevalent causes (as determined from hypothesis G – see figure 4.12) of death were plotted (figure 4.13). Other disease types are those for which there were fewer than four cases presented in necropsy reports and those for whom the cause of death that were 'unknown' or 'not applicable'.

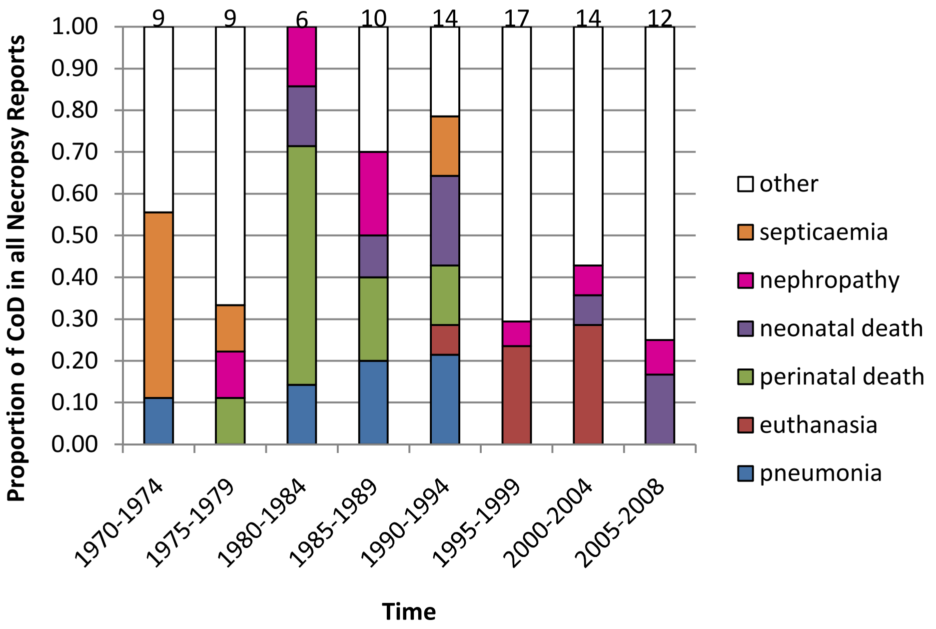

Figure 4.13. *Different diseases attributed to cause of death in all necropsy reports over time and the total number of necropsy reports written in each time category (values on top of the bars).*

There are no inherently obvious patterns to the frequency of disease over time in figure 4.13. Data did not meet the required conditions (see section 4.4.2.) for analysis of the observed frequencies of all disease types over time. It is not possible to comment on the hypothesis that there are trends in time in the prevalence of disease.

However, a *post hoc* analysis of the prevalence of individual diseases over time did exhibit differences. Table 4.2. illustrates the effect of time on the incidence proportion of the six diseases. Septicaemia exhibits a significant negative correlation with time (at the 0.001 level), and euthanasia a significant positive correlation with time (at the 0.05 level) with time.

Disease	ANOVA
septicaemia	0.001**
euthanasia	0.041*
perinatal death	0.065
neonatal death	0.380
pneumonia	0.640
nephropathy	0.797

*Table 4.2. Table to show the results for the most prevalent disease types and the ANOVA p values between a GLM of the data with a GLM of no variation (significance codes: ** = 0.01, * = 0.05).*

4.3.3. Hypothesis I: There is a relationship between the occurrence of different diseases and zoos

The proportion of deaths in each zoo that was attributed to the six most prevalent causes of death and the size of the deceased population were plotted (figure 4.14).

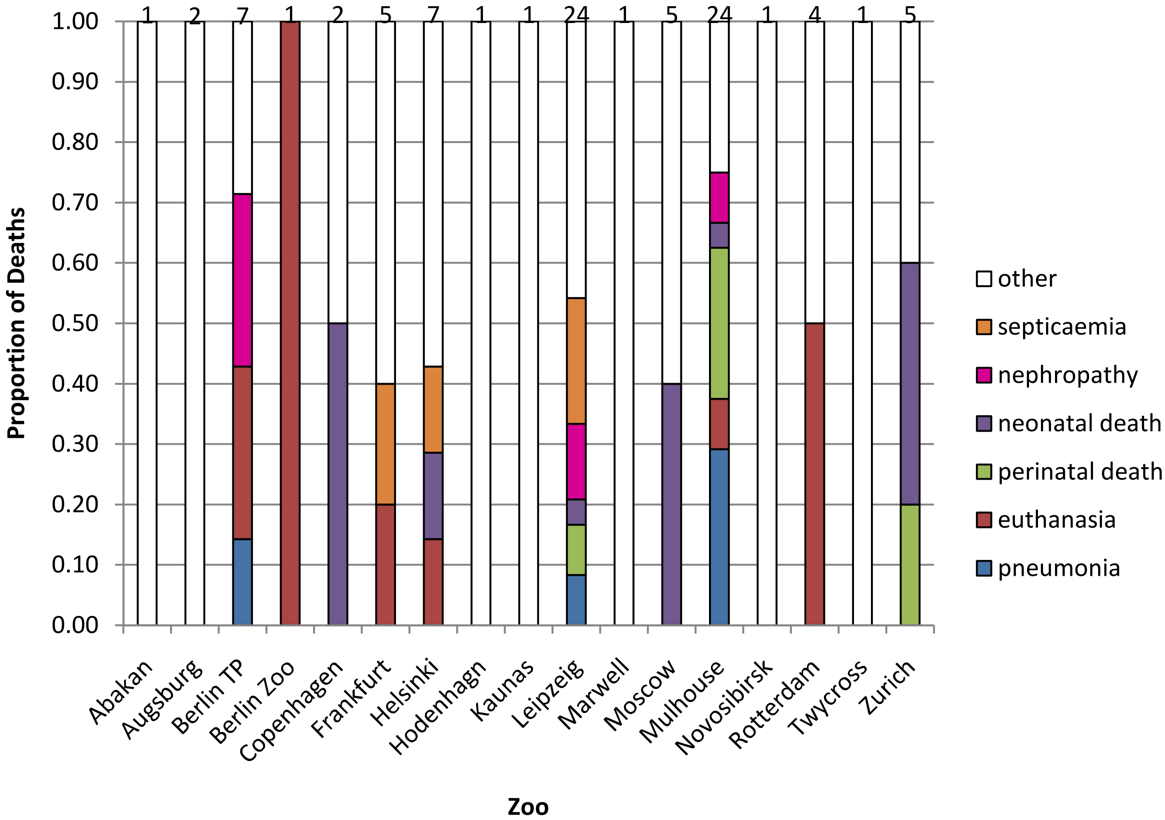

***Figure 4.14**. The proportion of different diseases attributed to the cause of death according to necropsy reports, and the total number of necropsy reports written for deceased cats in each zoo (value at the top of the bars).*

Figure 4.14 does not reveal any obvious patterns of fatal disease over time. Data did not meet the required conditions (see section 4.4.3.) for analysis of the observed frequencies of all disease types across all zoos that wrote necropsy reports. It is not possible to comment on the hypothesis that there are trends in time in the prevalence of disease.

4.3.4. Hypothesis J: There is a relationship between occurrence of different diseases and age at death

The three most prevalent disease processes (see section 4.3.1.) were graphed with the age class of each cat at the time of death (figure 4.15.).

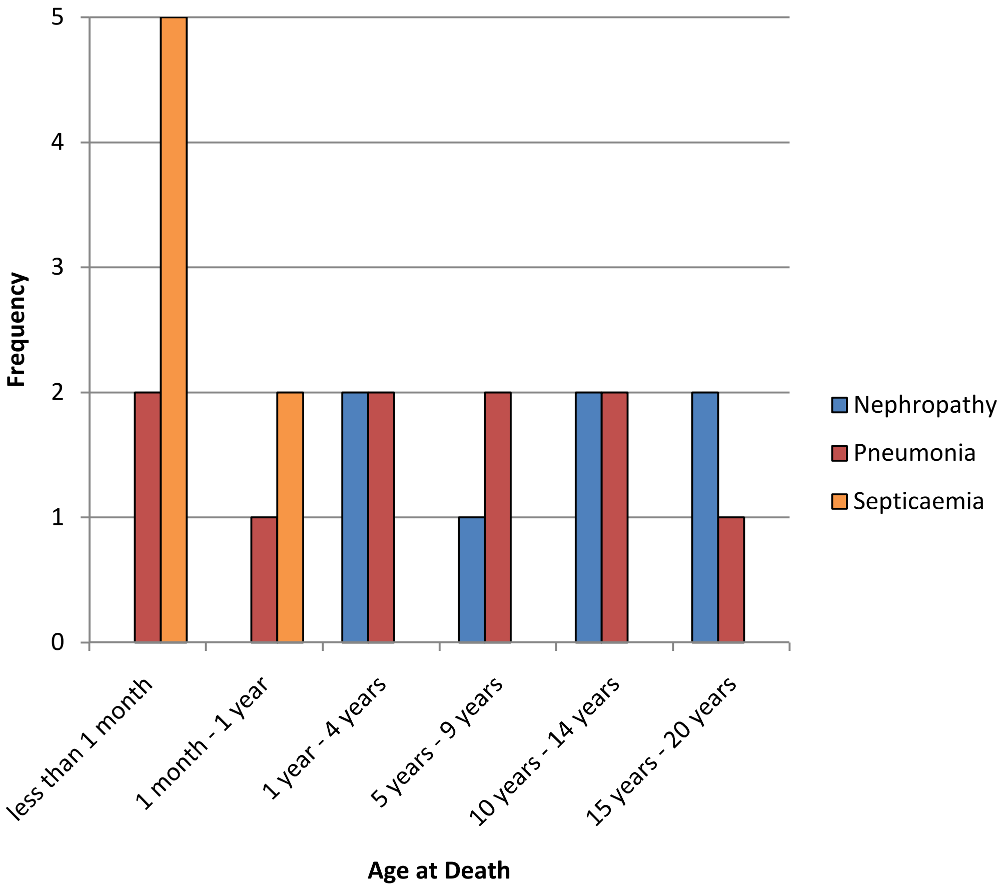

Figure 4.15. *Age at death and frequency of the most prevalent diseases.*

Nephropathy caused mortality in cats of over one year, pneumonia was a cause of death in cats of all ages and septicaemia was responsible for deaths in cats of less than one year only. Table 4.3. show that for nephropathy and septicaemia there are significant differences in prevalence with the age of the individual at deat.

Disease	ANOVA
nephropathy	0.011**
pneumonia	0.352
septicaemia	0.001***

*Table 4.3. Table to show the results for the most prevalent disease types with age and the ANOVA p values between a GLM of the data with a GLM of no variation (significance codes: ** = 0.01, *** = 0.001).*

A null hypothesis can be rejected, the hypothesis that there is a relationship between disease prevalence and the age of death.

4.4. Summary of Results

Objective	Relationship with time	Dependent on zoo	Zoos and time	Other
A: Extent of Necropsy Report Writing	(Hypothesis A) no	(Hypothesis B) yes	(Hypothesis C) no	relationship between the number of deaths in a zoo and proportion of deaths with a necropsy report
B: Quality of Necropsy Reports	(Hypothesis D) yes	(Hypothesis E) yes	(Hypothesis F) no	relationship between number of necropsy reports written and quality of necropsy report
C: Elementary Mortality Survey	(Hypothesis H) lack of data	(Hypothesis I) lack of data	N/A	(Hypothesis G) some diseases were significantly more prevalent than others (Hypothesis J) the prevalence of some diseases were significantly different in the age at death

Table 4.4. *Summary of objectives, hypotheses and results*

5. Discussion

5.1. The Database

Attempts were made by the author and J.L. to enable future integration of the database into the ZIMS software currently in development by ISIS. Data import from this database into ZIMS requires identical terminology and structure in the two programmes. A dialogue with ISIS staff members Rachael Thompson, Mike Kelly and Richard Langree was opened in order to discuss how integration may be possible. It emerged that ZIMS has not yet decided the terminology and structure to be used in the software. However, ISIS will ensure that the data stored in current MedARKS software can be integrated into ZIMS. By using MedARKS terminology in this database is hoped that data import of veterinary data once ZIMS is in operation will still be possible.

The database enabled efficient extraction of all data required for analysis. However, its potential reaches far beyond the needs of this thesis. Although only a portion of pathology data was required for analysis, the database has been designed to hold comprehensive veterinary information for every individual that is, or has been, in the AL-EEP. In response to the problems associated with ISIS databases, the delayed development of ZIMS and a bespoke database not freely distributed to others (see section 2.4.), this database has potential to be of significant value to captive population managers of a wide variety of species.

Prior to distribution to others, further work is necessary to refine this first version of the database. For example, the data entry form could be improved to increase ease of data input and minimise risks of inappropriate data entry or omission of important information. The author hopes to be involved in consultations with professional information technology personnel and J.L. to further the development of the database. The vision is for a working database that is freely available to all captive population managers.

5.2. Necropsy Reports

It is known that in at least 51% of all AL deaths, a necropsy examination was carried out. However, as necropsy reports have actually been written for only 37% of all deaths, it cannot be assumed that a necropsy report is available for all these examinations. The report may not have been written, or it may have been misplaced within the zoo. Out of 45 zoos, only 10 have submitted necropsy reports; although it is promising that over time there has been a noticeable increase in the quality of these reports. For a further 39% of

AL deaths, it is not known whether a necropsy examination even took place (table 4.1.). I tentatively suggest that there are a significant number of deaths that have not been necropsied. Figures collected show 54% of deaths lack a necropsy report; and for 9% a report is still outstanding (figure 4.1.). From the above it can be concluded that throughout the AL-EEP, very few zoos have been meeting their obligation to conduct necropsy examinations, write necropsy reports and submit them to J.L.

Over time, the percentage of deaths for which a necropsy report has been written has not changed significantly. However, a recent increase in the figures for 2005-2008 from the figure in 2000-2004 to is encouraging, although 60% is still less than ideal in light of the proposed reintroduction programme. Although it is encouraging that there is an increase in the number of necropsy reports submitted after an AL death, of potential concern is the reduction of necropsy reports with conclusive CoDs, dropping from 62% during 2000-2004 to 25% between 2005-2008 (figure 4.7). With the improvement of tools, technology and knowledge of pathology since 1970s, an increase in the confidence of CoD diagnoses could have been expected. In future, in order to have a high proportion of good quality necropsy reports, effort is needed to focus on how to improve the content of that report.

Between zoos there are significant differences in proportion of deaths for which a necropsy report is written over time (figure 4.6) and the proportion of necropsy reports that have a conclusive CoD over time (figure 4.11.). For the extent of necropsy report writing and quality of report written, different zoos do not demonstrate similar behaviour over time, but instead behave independently of one another and that there are other factors that influence zoo behaviour.

5.3. Factors Influencing Zoo Behaviour

It is likely that there are many more factors that influence the behaviour of zoos: a) to perform a necropsy examination, b) to write a necropsy report and c) to provide a high quality report. *Post hoc* analyses suggest that number of deaths at each zoo may partially explain the behaviour of zoos in writing necropsy reports and the quality of reports written (see figures 4.4. and 4.9.).

The number of deaths at a zoo is indicative of the level of involvement of that zoo in the AL-EEP. As a zoos' involvement with the AL-EEP increases, perhaps the increased communication with S.C., T.A. and J.L. encourages submission of data. The zoo may also be placing more importance or a higher priority on the AL within the collection. Despite the observed relationship with the number of deaths at a zoo with proportion of deaths with a necropsy and conclusive CoDs, there are limitations to using quantitative proportions and frequencies to investigate and explain the behaviour of zoos in the AL-EEP.

When taking a quantitative look at behaviour of zoos, if the background information to the situation is overlooked then it is easy to reach an incorrect conclusion. For example, at first glance the behaviour of Prague is of concern (see section 4.1.3.), there is very little information about the mortality of five cats that have died since as recently as 2001. However, further investigation reveals that three cats (from the same litter) that died in 2002 were newborn cubs that had been eaten by the mother. This demonstrates the hazards in using simplistic quantitative analyses and extracting crude frequencies of this data.

Zoos involved in the AL-EEP are considerably heterogeneous. Across western Europe, eastern Europe and the Russian Far East such regions resources available to zoos will vary significantly, thus affecting the extent to which they are able to collect data in the first instance and then submit to J.L..

In addition between the death of an AL. and J.L. receiving a necropsy report, a specific process has to occur (see figure 5.1.). A break in any part of the chain-of-events will result in failure to provide a necropsy report to J.L..

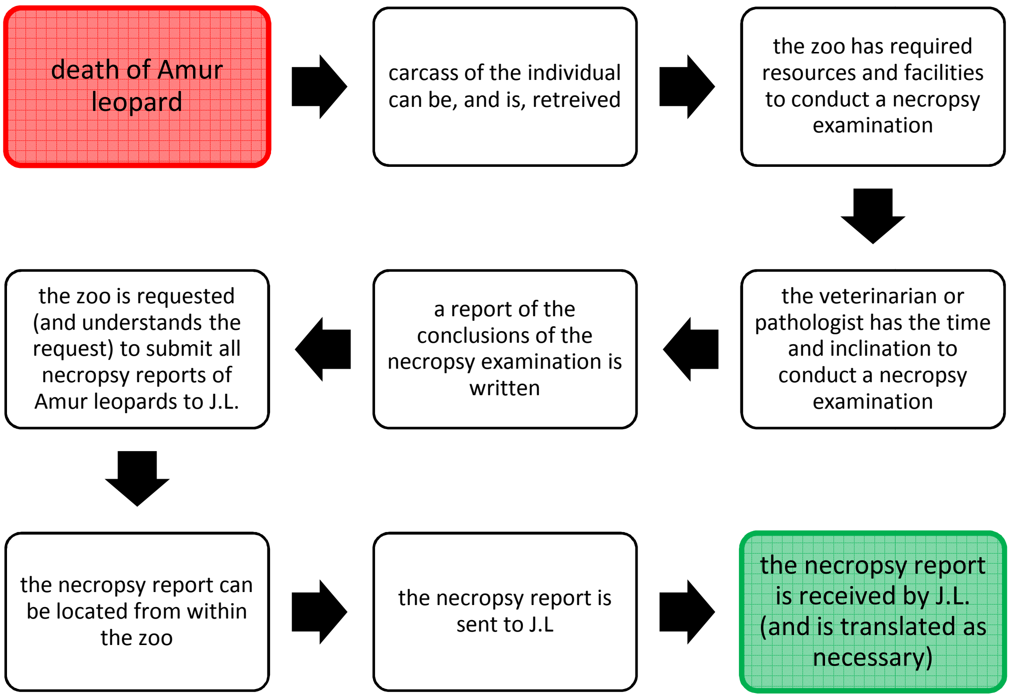

Figure 5.1. *Process from death of an Amur leopard to J.L. receiving the necropsy report.*

In describing the chain-of-events required for J.L. to receive a necropsy report it is possible to see how social, political and economic factors could cause a breakdown in any aspect of the chain which prevents a necropsy report getting to J.L.. Possible issues include:

- *carcass not retrieved:* eaten by another animal;
- *lack of facilities:* the zoo has very limited veterinary facilities;
- *lack of veterinary resources:* veterinary department may be over-stretched so that a necropsy examination does not take place or a report is not written;
- *language barrier:* although English is the working language of the EEP, English has not been taught in all schools throughout Europe, zoo staff may not speak English nor understand requests for pathology information;
- *communication difficulties:* the zoo does not send a representative to EAZA conferences and is not aware of the value of necropsy reports (see section 3.1.);
- *political restrictions:* during the Soviet era, zoos within the Soviet block may not have been able to report 'bad' news to captive population managers in the west.

Considering the vast differences between zoos involved in the AL-EEP it is not surprising that the behaviour of these zoos cannot be quantitatively explained. Since the 1960s when the first AL was brought into captivity there have been monumental political changes – such as the breakdown of the Soviet Union and expansion of the EU. Zoos have not been exempt from changes that have occurred during this time. However, effort should focus on the means by which the extent of necropsies conducted and good quality reports written can be improved in the very near future.

5.4. Increasing Quantity and Improving Quality of Necropsy Reports

Two very different approaches to increasing the proportion of deaths that are investigated and a necropsy report submitted to J.L. could be taken: 1) Restricting the entire captive population to only those zoos that have a good track record in submitting high quality necropsy reports. 2) Understanding and responding to these problems that make submission of a necropsy report problematic. Considering that the politics and logistics of the former are unfeasible (e.g. remove all captive Amur leopards from Russian zoos – their country of origin!), the second approach is the only viable option.

Existing pressure on zoos from J.L., S.C. and T.A. to collect and record veterinary data needs to be sustained and expanded (where possible). Visiting each zoo in the AL-EEP to run workshops to improve necropsy examination and report writing skills is likely to improve compliance. However, in the short term financial resources are likely to limit this option. There is increasing recognition of the roles that zoos can play in conservation, it is hoped that there will be an increase in financial investment to develop the capacity of zoos to contribute to conservation projects. However, in the interests of a health assessment of the AL-EEP and as a component of the AL reintroduction programme it is crucial that zoos failing to meet obligations to collect and submit data are encouraged to do so. Zoos could be incentivised to produce necropsy reports, for example payment for each necropsy report received. Perhaps there could be 'naming-and-shaming' of zoos amongst the European zoo community, with the threat of removal of AL for non-compliance.

Despite being repeatedly told by J.L. at conferences, in reports and emails since 2002 (see section 3.1.), some zoos may still not understand the value and importance of pathology data from the Amur leopard EEP. Presenting figure 4.3. to AL-EEP zoos to show the low level of veterinary support across the AL-EEP may jolt them into increasing the proportion of deaths for which there are necropsy reports in the future.

5.5. Elementary Mortality Survey

5.5.1. Disease Prevalence

A variety of diseases cause mortality in the Amur leopard EEP, although there are specific diseases that result in significantly more mortality than others (figure 4.12). The most prevalent diseases are: pneumonia, nephropathy and septicaemia (figure 4.16). Of these diseases only pneumonia was observed across all age classes, whilst septicaemia was responsible for mortality in only young cats, and nephropathy only in cats one or more years old at the time of death.

Only septicaemia and euthanasia showed any significant differences in their prevalence over time. Septicaemia was significantly more prevalent during 1970s-1990s than in recent times, the converse is true of euthanasia which was not identified as a cause of death until 1990. Disease prevalence does not seem to be related to certain zoos as all six of the most prevalent diseases have been seen in at least two or more zoos.

An accurate evaluation of the prevalence of different diseases over time and between zoos requires substantial increase in the proportion of deaths for which there is a good quality necropsy report. However, these elementary statistics can be compared with mortality surveys from other captive big cat populations to determine if the Amur leopard EEP suffers from identical problems, and if so, can similar veterinary care (treatment and or prevention of disease) can be applied between the different species.

5.5.2. Comparison of Amur Leopard Disease Prevalence with Other Captive Big Cat Populations

The prevalence of the most frequently occurring diseases and the age at which deaths occurred in the Amur leopard EEP are compared with mortality surveys of other captive big cat population. The populations of captive big cats for which comprehensive mortality surveys have been published in available journals are 1) the jaguar (*Panthera onca*) in North America by Hope and Deem in 2006 and 2) of cheetah (*Acinonyx jubatus*) SSP by Munson in 1993 (see section 2.4.1.).

Pneumonia has caused mortality of cats of all ages throughout the Amur leopard EEP. In captive jaguar of North America pneumonia was also identified as a cause of death in cats of various ages (Hope and Deem 2006). In contrast, in the pathology survey of captive cheetah pneumonia was the most prevalent disease in cubs of one to sixteen days old (Munson 1993).

Nephropathy (kidney disease) has occurred throughout the history of the Amur leopard EEP, but only in cats of one year old or more. Mortality relating to kidney disease increased with age of jaguar in captivity (Hope and Deem 2006), and in 26% of all deaths in the North American captive cheetah population (Munson 1993).

There is a high prevalence of septicaemia amongst leopards younger than one year, although it has not been observed since 1975, except for two cases at two separate zoos in 1992. Munson (1993) describes one case of septicaemia in cheetah, also in an individual less than sixteen days old, although there is no occurrence of septicaemia in any jaguar in the North American captive population (Hope and Deem 2006).

In the AL-EEP, 59% of leopards died before the age of 2 years. This is substantially higher than 24% of the North American jaguar that die before reaching 2 years between 1982-1992 (Munson 1993; Hope and Deem 2006). Munson (1993) define cheetah cubs as being less than 16 days old, for which there is a death rate of 24%, which is in the same scale also the AL-EEP of 33%.

Overall there are some similarities with the Amur leopard EEP and other captive big cat populations, such as the prevalence of pneumonia in younger cats and nephropathy in older cats. However, the Amur leopard seems to have a substantially higher prevalence of septicaemia than in captive jaguar and cheetah populations in North America, and a higher juvenile mortality of the jaguar population, although of the same scale to that of the cheetah population.

5.6. Strengths and Limitations of the Thesis

There is a limitation in using the confidence of the CoD to grade the quality of necropsy reports. There may be circumstances where a particular disease may be difficult to diagnose, although a thorough necropsy examination was conducted and a detailed report written. There may also be instances where a number of diseases are present in an individual at the time of necropsy, each could have equally been the CoD, making it difficult to be certain about the true CoD. In future, perhaps a better way to grade the quality of a necropsy report is to score the report on a number of criteria such as the number of pathology tests conducted, details on the type of necropsy performed (e.g. partial, full), areas of the specimen investigated, extent to which the AL-EEP necropsy protocol was adhered to, whether samples were taken and stored to aid further research attempts.

Problems associated with the availability of pathology information from ISIS database were overcome by collection of necropsy reports directly from zoos and standardisation of data input into a different database. This thesis is thought to be the first assessment of the behaviour of zoos in meeting obligations as holders of EEP specimens and contributing to conservation strategies.

The ability of the author to perform the required statistical tests was confounded by the lack of data available, and so the thesis is only able to provide an elementary mortality survey. However, the database has created the mechanism to enable further investigation and with veterinary expertise it is likely that a mortality survey can be created.

In addition, the database will facilitate the work of J.L. and enable future research of the Amur leopard EEP to create normal values for screening and sampling data and to and assist construction of a comprehensive mortality survey. The database has potential to enable further investigation of the behaviour of zoos (see section 5.7.).

5.7. Future Directions

This thesis only scratches the surface in terms of assessing the behaviour of zoos in contributing to conservation strategies. Rather than providing succinct answers to the research questions set out in section 1.5., this analysis opens up more questions and future directions for database development, extent of necropsy examinations, necropsy report writing amongst zoos, mortality survey of the Amur leopard EEP and an overall assessment of the behaviour in zoos, some of which are described below.

5.7.1. Database

- Professional IT expertise and funding needs to be sought to improve the database developed as part of this thesis.
- Incorporation of data from the examination of wild AL to allow direct comparisons between wild and captive counterparts.
- Data entry of bio-sample, clinical and screening information to facilitate research and generation of normal values specific to the taxon.
- Once the database is fully functional and meets the needs of captive population mangers, every effort should be made to distribute the database to other practitioners and to encourage modification of the database for its improvement.
- Provision in the database for all previous locations and the current location for every cat. Spatial analyses could include: a) investigation of the pattern of movements of cats between zoos and epidemiology, b) identification of the location of an individual to clinical and morbidity information, and c) investigation of the effect of moving cats between zoos on health of the individual.

5.7.2. Necropsy Report Writing

The effect that the following characteristics have on necropsy examinations and the production of good quality necropsy reports:

- financial investment/input;
- zoo size – physically and the number of species held;
- veterinary resources and facilities;
- attendance of zoo representative to EAZA conferences;
- extent of English understood and spoken in the country and/or by zoo personnel especially veterinarians and pathologists;
- historical and political issues affecting zoo activities;
- extent of knowledge of the importance to the AL-EEP and reintroduction programme of the individual/s held by the zoo;
- conservation ethos of the public in the country and of the zoo.

- The true extent of zoos meeting their obligations to the Amur leopard EEP should be presented to zoos at conferences and in papers in order to demonstrate to them the shortfall in pathology information available. Although there has been pressure on zoos from J.L., S.C. and T.A. to produce more data – it is hoped that presenting zoos the facts in 'black-and-white' will a) stimulate activities and b) open a dialogue from which to discuss and investigate the problems in compliance and possible solutions to them.

5.7.3. Mortality Survey

- The use of veterinary expertise and knowledge for a comprehensive interpretation of the significance of diseases prevalence to inform appropriate veterinary management for the AL, other leopard subspecies and different taxon.
- Analysis to discriminate between preventable diseases and those inherent in the AL.
- Analysis of clinical observations, related and incidental pathology in the same was as CoD analysis and mortality surveys.
- Differences in disease between males and females.
- Prevalence of multiple types of disease with age.
- Associations of disease with reproductive success.
- Concurrence of diseases between CoD, related and incidental pathology.
- Screening results with disease prevalence.
- Further investigation of the relatively high prevalence of septicaemia compared with population of other captive big cats.
- Further investigation of the causes of peri- and neo-natal death in AL.
- To build a family tree on which disease could be mapped and analysed for relationships with genetic lineages.

5.7.4. Investigation of the Behaviour of Zoos

- Further research on the factors influencing zoo behaviour to producing necropsy reports should be coupled with increased investment and an awareness of the value of such data.
- The production and submission of pathology information of the Amur leopard EEP towards a mortality survey of the population is used as a case study of the behaviour of zoos. However, the behaviour of zoos towards a charismatic big cat is likely to be very different from that of a small reptile, amphibian or insect species. To further the investigation of species bias within zoos, this study could be widen to include and compare between different taxa.
- Investigation of the contribution of captive populations in other regions to conservation strategies – such as North American Population Management Programmes (PMPs) and Species Survival Plans (SSPs).

5.8. Conclusion

With reference to the research questions set out in sections 1.5.2., 1.5.3. and 1.5.4., the following conclusions can be drawn from this thesis:

1. Overall AL-EEP zoos are not meeting obligations to do necropsy examinations, write good quality necropsy reports and submit them to J.L.. It cannot be assumed that a necropsy report is written for every necropsy examination.
2. The proportion of deaths for which there is a necropsy report has not significantly changed over time (from 1970s to 2008).
3. Some zoos are significantly better at conducting necropsy examinations, writing necropsy reports and submitting them to J.L. Over time and between zoos there is no trend in the proportion of deaths for which there is a necropsy report. Zoos behave independently of one another.
4. The quality of necropsy reports has improved since the 1970s.
5. Some zoos produce better quality necropsy reports than others.
6. There is insufficient data on the mortality of AL for a comprehensive and accurate analysis, although an elementary mortality survey can guide future research directions.
7. Pneumonia, septicaemia and nephropathy are the most prevalent diseases in the AL-EEP. The population also has a high proportion of perinatal and neonatal deaths, a high occurrence of euthanasia and a large number of instances where the CoD cannot be established.
8. The prevalence of septicaemia has significantly reduced since 1990. The occurrence of euthanasia has significantly increased since 1990.
9. Although a lack of data prevented a comprehensive analysis, disease prevalence does not seem to be related to zoos as each of the six most prevalent diseases were observed in two or more zoos.
10. There is a significantly higher prevalence of septicaemia in cubs under one year old, and nephropathy in adults than in leopards of all other ages.
11. The high prevalence of neonatal, perinatal, pneumonia and necropsy in the AL-EEP were also observed in captive jaguar and cheetah populations in North America. The high prevalence of septicaemia seen in the AL-EEP seems uncommon amongst jaguar and cheetah.

Word Count = 13, 919

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Appendix 1

Example of a comprehensive necropsy report.

IZVG Pathology
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07-0081

IZVG number:	07-0081	**Owner:**	Twycross Zoo Park - East Midlands Zoological Society Ltd
Vet submitting sample: Nic Masters		**Animal ID:**	Izy 4156
		Species:	Amur leopard ISBN: 560 Panthera p. orientalis
Date received:	19/01/2007	**Age:**	4 years 6 months
Date reported:	23/01/2007	**Sex:**	Female

Brief clinical summary:
Has been off colour 2 weeks, began with loss of appetite and progressed to general dullness. Some response to potentiated amoxycillin, but became depressed again on morning of death with laboured breathing. Has nearly-weaned cubs 8 weeks old. Midline hole/tract noted at umbilicus (?congenital), swab taken previously.

Gross Post Mortem Report

Description:
Species: Adult female Amur leopard.
Weight: 45.1 kg.
Body condition: Good. Plentiful fat stores are present in the subcutaneous tissue, and throughout the mesentery, mediastinum and retroperitoneal areas.

Skin/subcutis: There is a solitary slit-like perforation (approximately 1 cm diameter) into a subcutaneous blind ending fistula, situated in the ventral midline at or very near to the normal position of the umbilicus. This is surrounded by an area of alopecia. Incision into the fistula identifies a saccular fibrous encapsulated tract, approximately 7 cm long, with a roughened red lining, accumulations of green/yellow purulent debris, and several embedded fragments of a woody stem of a plant (bamboo?). This is encapsulated within the subcutaneous tissue, and is not continuous with the peritoneal cavity at this time.

Digestive system:
Teeth and tongue: Teeth are in good condition, and tongue is unremarkable.
Oral cavity: Unremarkable.
Oesophagus: Unremarkable.
Stomach: The stomach is empty, with surface mucus and focal ecchymotic haemorrhages of the cardiac mucosa.
Small intestines: Contain unremarkable creamy haemoglobin stained content.
Large intestines: Large intestines contain plentiful amounts of rather dry crumbly faecal material.
Liver: The liver has smooth capsular surfaces and slightly rounded lobe margins. Colouration is pale tan to yellow, with a soft greasy cut consistency. Gallbladder is distended. Embedded in the fat of the falciform ligament and associated with a cluster of lymph nodes, are fragments of stems of a woody plant (up to approximately 30 mm long), that are embedded in a partial capsular fibrous tissue and accumulations of green/yellow purulent debris.
Pancreas: Soft, purple/pink.
Abdominal cavity: There is a marked excess (approximately 200 ml) of dark red slightly turbid watery fluid. Omental and mesenteric serosal surfaces are markedly hyperaemic, with indistinct cream granular roughening. There is an erosion into a small omental blood vessel, with an adherent firm thrombus (approximately 6 cm diameter) and surface traces of fibrin.

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Respiratory system:
Nares/nasal cavity: Unremarkable external nares. Nasal cavity is not opened.
Larynx/trachea: Unremarkable.
Lungs: Both lungs are partially collapsed, dark red, with rubbery, slightly heavy consistency, and wet dark red cut surfaces that ooze watery fluid. Float in fixative.
Pleura: There is a marked excess (approximately 300 ml) of dark red slightly turbid watery fluid. Pleural and mediastinal serosal surfaces are hyperaemic and there is indistinct cream granular roughening, particularly over the mediastinal surfaces.

Cardiovascular system:
Heart: Mild excess of pericardial fluid. Ventricles, atria, valves and myocardial cut surfaces are unremarkable.
Major vessels: Unremarkable.

Haematopoietic/lymphoid system:
Spleen: Predominantly smooth capsule with grey sheen and occasional marginal nodules of siderocalcinosis. Cut surfaces are slightly tough, dark red.
Lymph nodes: Typically small, soft, with cream cut surfaces. Mild oedema in hepatic nodes.
Thymus: Not identified.
Bone marrow: Small amounts of red rib marrow are present, femoral bone marrow is not examined.

Urinary and genital systems:
Kidneys: Capsules strip readily from both kidneys reveal smooth cortical surfaces. Distinct demarcation between paler brown cortex and darker red medulla on cut surfaces. Unremarkable pelvis.
Ureters: Unremarkable.
Bladder and urethra: Bladder is empty apart from a small amount of viscous yellow/cream turbid mucoid fluid. Mucosal surfaces are cream and wrinkled. Unremarkable urethra.
Gonads and genitalia: Paired firm cream symmetrical ovaries. Uterus is fully involuted, with cream tightly contracted uterine horns and body, containing a minimal amount of slightly green tinged mucoid debris. Unremarkable cervix. Mammary glands are dark pink and moist when incised, yielding scant droplets of milk on the cut surfaces.

Nervous and musculoskeletal systems:
Brain: Not examined, in order to preserve the skeleton for later museum use.
Spinal cord: Not examined.
Peripheral nerve: Unremarkable sciatic nerve.
Muscles: Unremarkable.
Bones: Not examined in detail, no significant abnormalities noted.
Joints: Not examined in detail.

Endocrine system:
Adrenals: Unremarkable corticomedullary demarcation on cut surfaces.
Thyroid/parathyroids: Unremarkable dark brown firm paired thyroids.
Pituitary: Not examined.

Microbiology:
Abdominal fluid: A sparse growth of non haemolytic E. coli is isolated in aerobic culture.
Pleural fluid: No bacteria are isolated after 72 hours incubation in aerobic culture.

No bacteria are isolated from either abdominal or pleural locations by anaerobic culture.

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Bladder swab: Sparse growths of non haemolytic E. coli and Streptococcus are isolated in aerobic culture.

Gross diagnoses:

1. Severe serosanguinous to suppurative pleuritis and peritonitis, with intra abdominal plant foreign bodies and omental haemorrhage, thoracic and abdominal cavities.
2. Moderate partial acquired atelectasis, lungs.
3. Focally extensive chronic subcutaneous fistula, with intralesional plant foreign bodies, umbilical skin.
4. Suppurative cystitis (?), urinary bladder.
5. Lipidosis, liver.
6. Minimal focal mucosal haemorrhage, stomach.
7. Minimal siderocalcinosis, spleen.

Comment:

The cause of death is likely to be respiratory insufficiency as a result of lung collapse due to the expanding volume of pleural and peritoneal fluid, complicated by bacterial toxaemia. The underlying reason for the accumulation of these fluids is a penetrating plant foreign body, fragments of which are present in the peritoneal cavity adjacent to the liver in the region of the falciform ligament. Similar material is present in the subcutaneous fistula located in the umbilical region. The plant has woody stems and resembles bamboo, with large fragments up to approximately 2 cm long being embedded in both the cutaneous fistula and the peri-hepatic connective tissues. Migrating plant foreign bodies of this sort are not uncommon causes of peritonitis and pleuritis in carnivores, more typically associated with migrating grass seeds, and often with bacterial infections involving either Actinomyces or Nocardia. In this case, neither of these organisms is isolated from either pleural or peritoneal fluid, but since the animal has been under antibiotic treatment, the isolates may not be the most clinically important ones. Histological examination may help to clarify whether the changes support a role for either of these organisms.

Lipidosis in the liver is likely to be a secondary development in a toxaemic animal with ample fat stores.

The significance of the pus-like material in the bladder is uncertain. There are no convincing gross changes in the bladder mucosa.

Siderocalcinosis is an incidental finding, resulting from mild deposits of iron and calcium in the capsular connective tissues of the spleen.

The mammary glands of this animal are currently producing very small amounts of milk, implying that the cubs may be deriving most of their food from alternative sources at this stage.

Histological examination of selected tissues will be undertaken, and a further report issued.

Histology Report

Description:

Pancreas, thyroid/parathyroid: Unremarkable.

Adrenal: There is pronounced congestion at the junction between cortex and medulla. Occasional small nodules of well-differentiated cortical cells bud into the capsular surface.

Stomach: There is multifocal hyperaemia of the superficial mucosa.

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Kidney: There are minimal interstitial foci of lymphocytes and plasma cells.

Urinary bladder: There is autolytic detachment of the urothelium. A few red blood cells are also present within the luminal debris, along with occasional rod-shaped bacteria without tissue reaction.

Heart (left ventricle): There is plentiful epicardial fat. Occasional minimal foci of fibrosis with a few atrophic muscle fibres and very small numbers of perivascular lymphocytes are present in the outer ventricle.

Spleen: Mildly congested red pulp, intermingled with small numbers of haematopoietic cells. Lymphoid sheath has discrete follicles with little germinal activity.

Liver: Overall architecture is unremarkable, although there is autolytic hepatocyte dissociation. Patchy multifocal sinusoidal congestion is present. Clusters of hepatocytes throughout the section have mild vacuolar change. The hepatic capsule is thickened by a layer of organising fibrin, intermingled with red cells, small numbers of neutrophils, macrophages, reactive fibroblasts, and a basal infiltrate of lymphocytes and plasma cells. There are small numbers of mixed, predominantly large rod-shaped bacteria within the capsular debris.

Omentum: A sheet of haemorrhagic debris intermingled with foci of neutrophil and macrophage accumulation.

Mesentery and mediastinal connective tissues: Both are similar, with diffuse vascular hyperaemia and sprouting capillaries with swollen endothelia. Some vessels contain fibrin thrombi. Intervening connective tissue stroma has a diffuse mixed infiltrate of neutrophils and plump foamy reactive macrophages, as well as small numbers of lymphocytes and plasma cells. Significant numbers of large rod-shaped bacteria are also intermingled in the debris.

Lung (two sections): Both are similar. There is widespread alveolar oedema, congestion and collapse, with frequent megakaryocytic entrapment within septal capillaries. Pleural surfaces are variably thickened, with a surface layer of fibrin and red cells, interspersed with small numbers of neutrophils and larger numbers of plump foamy macrophages. Mesothelial cells are variably denuded, but where present are hyperplastic and occasionally multinucleated. There is variable subjacent organising granulation tissue and fibrosis of the pleura, with intermingled populations of lymphocytes and plasma cells. Populations of similar large rod-shaped bacteria are scattered in the inflammatory debris. Increased populations of alveolar macrophages are present in the peripheral alveoli beneath the thickened pleura.

Umbilical skin: Unremarkable haired skin gives way to a sac-like invagination of inflamed granulation tissue. A central cavity contains neutrophils and fibrinonecrotic debris, and the surrounding tissue is diffusely oedematous, with numerous dilated swollen branching capillaries interspersed with pockets of fibrinonecrotic material with neutrophils, and by variably mature fibrous tissue. The latter extends into the surrounding dermis and effaces the majority of the hypodermal adipose tissue. A further section includes similar neutrophil-inflamed granulation tissue with a central lumen containing fragments of plant material. The surrounding fibrosis blends with a lobule of hyperplastic mammary gland, in which there is a small amounts of ongoing secretory activity.

Diagnoses:

1. Severe chronic-active fibrinosuppurative pleuritis and peritonitis, with intralesional rod-shaped bacteria, pleural and peritoneal cavities.

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2. Chronic cutaneous/subcutaneous fistula, with active suppurative inflammation and intralesional plant debris, umbilical skin.
3. Secondary atelectasis, lungs.
4. Minimal multifocal fibrosis, heart.
5. Mild nodular cortical hyperplasia, adrenal.

Comments:

The umbilical lesion is a chronic one, centred around plant debris, and comprising sheets of inflamed granulation tissue and fibrosis that extend irregularly into the surrounding skin and subcutaneous tissues. There are no clear anatomical landmarks to differentiate between a pre-existing congenital anomaly that has been secondarily contaminated with plant material and a primary tissue reaction to a penetrating foreign body. On balance, I suspect that the latter is probably more likely.

The pleuritis and peritonitis are consistent with an inflammatory reaction to a persisting bacterial infection secondary to the penetrating plant foreign body embedded near the liver. There is consequential collapse of the lungs as a result of the pleural fluid accumulation. The appearance of the bacteria and the reaction to them are not typical of either Nocardia or Actinomyces, and a opportunistic infection by another agent is suspected. No anaerobes were isolated, but the antibiotic treatment which this animal received prior to death may well have inhibited representative bacterial cultures.

The minimal changes in the heart and adrenal are considered incidental.

There is no evidence of either active infection with Toxoplasma, or of quiescent tissue cysts.

Tissue disposition:

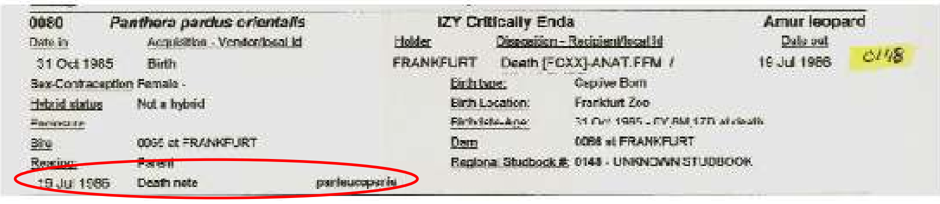
Range of tissues retained in formalin archive.
Liver, lung, spleen, kidney retained frozen at - 80°C.
Carcass retained frozen for submission to National Museums of Scotland.

Pathologist: M.F. Stidworthy MA VetMB PhD MRCPath MRCVS 07-0081

Appendix 2

Examples of ARKS comments, the mortality information is circled in red.

Example A.



0080 *Panthera pardus orientalis* IZY Critically Enda Amur leopard

Date in	Acquisition - Vendor/local Id	Holder	Disposition - Recipient/local Id	Date out
31 Oct 1985	Birth	FRANKFURT	Death [FCXX]-ANAT.FFM /	19 Jul 1986

0148

Sex-Contraception Female -

Hybrid status Not a hybrid

Birth type: Captive Born

Birth Location: Frankfurt Zoo

Birthdate-Age: 31 Oct 1985 - 0Y,8M,17D at death

Sire 0095 at FRANKFURT

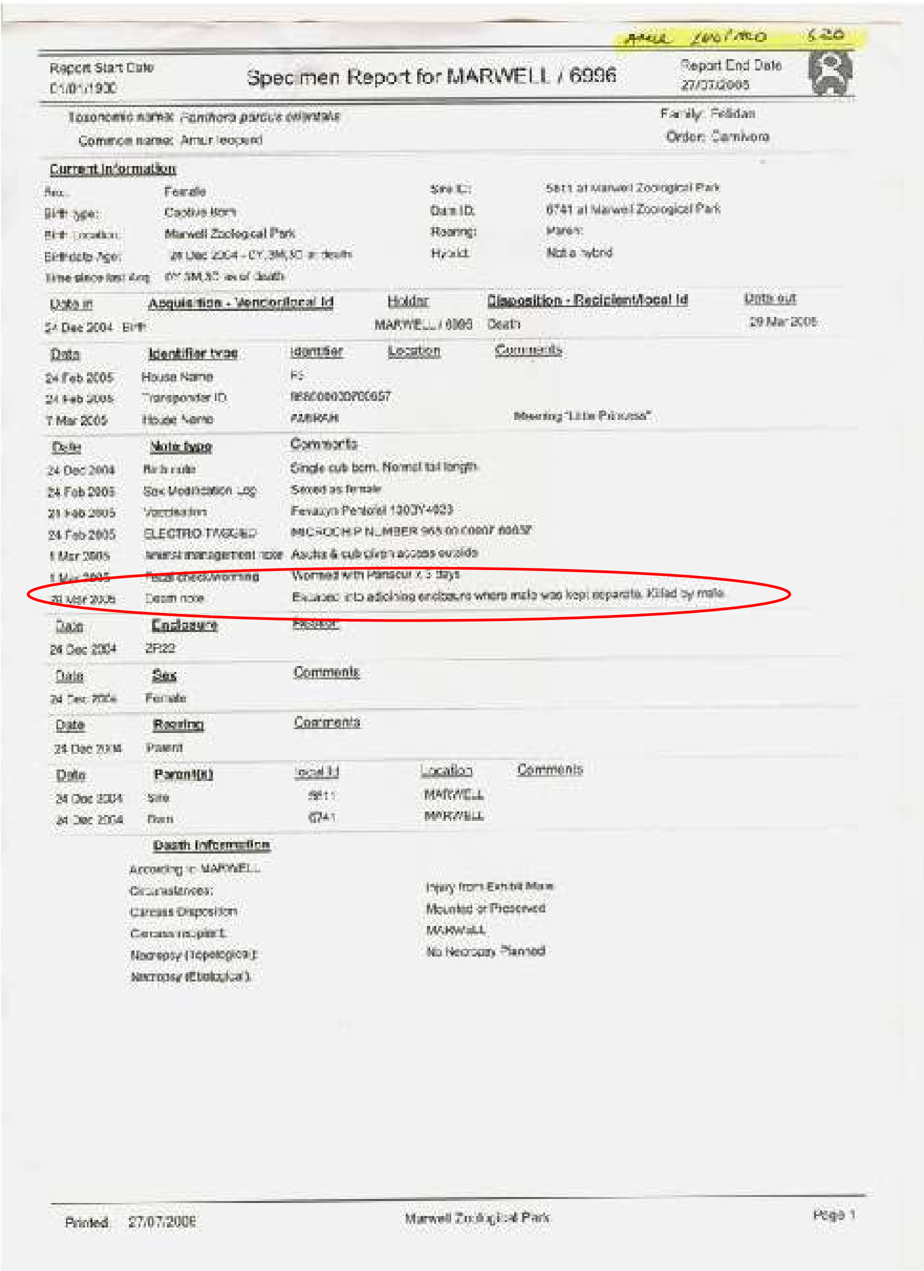
Dam 0088 at FRANKFURT

Rearing: Parent

Regional Studbook #: 0148 - UNKNOWN STUDBOOK

19 Jul 1986 Death note parleucoperis

Example B.



Report Start Date 01/01/1900

Specimen Report for MARWELL / 6996

Report End Date 27/07/2005

Taxonomic name: *Panthera pardus orientalis* Family: Felidae

Common name: Amur leopard Order: Carnivora

Current Information

Sex: Female Sire ID: 5811 at Marwell Zoological Park

Birth type: Captive born Dam ID: 6741 at Marwell Zoological Park

Birth Location: Marwell Zoological Park Rearing: Parent

Birthdate Age: 24 Dec 2004 - 0Y,3M,5D at death Hybrid: Not a hybrid

Time since last Acq: 0Y,3M,5D as of death

Date in	Acquisition - Vendor/local Id	Holder	Disposition - Recipient/local Id	Date out
24 Dec 2004	Birth	MARWELL / 6996	Death	29 Mar 2005

Date	Identifier type	Identifier	Location	Comments
24 Feb 2005	House Name	F5		
24 Feb 2005	Transponder ID	965000030700057		
7 Mar 2005	House Name	AMIRAH		Meaning "Little Princess"

Date	Note type	Comments
24 Dec 2004	Birth note	Single cub born. Normal tail length.
24 Feb 2005	Sex Modification Log	Sexed as female
24 Feb 2005	Vaccination	Fevaxyn Pentofel 1303Y-923
24 Feb 2005	ELECTRO TAGGED	MICROCHIP NUMBER 965 00 00007 00057
1 Mar 2005	Animal management note	Adults & cub given access outside
1 Mar 2005	Fecal check/worming	Wormed with Panacur x 3 days
29 Mar 2005	Death note	Escaped into adjoining enclosure where male was kept separate. Killed by male

Date	Enclosure	Reason
24 Dec 2004	ZR22	

Date	Sex	Comments
24 Dec 2004	Female	

Date	Rearing	Comments
24 Dec 2004	Parent	

Date	Parent(s)	local Id	Location	Comments
24 Dec 2004	Sire	5811	MARWELL	
24 Dec 2004	Dam	6741	MARWELL	

Death Information

According to MARWELL

Circumstances:	Injury from Exhibit Mate
Carcass Disposition	Mounted or Preserved
Carcass recipient:	MARWELL
Necropsy (Topological):	No Necropsy Planned
Necropsy (Etiological):	

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Appendix 3

Example of a note from zoo to J.L.

Example A.

Email 19-Sep-06: Nikolaev Zoo

Dear John Lewis,
I'd like to beg your pardon for some incorrect information sent to you in the previous message, that is not my fault.Please make the necessary corrections according to the present message:

We inform you that in Nikolaev zoo we had four posterities (births) from the amur leopards: a male #213 and a female #237. Each time it was two cubs. One cub from every three posterities had died, and each time earlier and earlier:

1.F363 Dulsina died of the lungs emphysema, pneumonia, nephritis, splenitis 08 June 1995;

2.M423 Paris died of the liver cyrrhosis 26 May 1996;

3.F462 was born nonviable and died 16 June 1997;

From the 4th posterity died both:

M475 died 04 February 1998 from pneumonia, as the mother did not feed it and was not warming him..

F476 was stillborn 03 February 1998.

From that time and up till now the male and female copulated regularly but got no posterity.

Best regards,
Tatiana Bondarenko
Curator carnivora

Example B.

(Email from Dr Liba Vesela, Olomouc Zoo, 19-Mar-08)

Dear Dr Lewis,

Thank you very much for your message, we will be pleased very much to be in a contact with you.

As far as the cubs born 12 Oct 2005 (660 and 661): We can´t send you any post mortem report as the cubs were not examined. The birth of the young female was expected, but the cubs were found in the morning dead. Probably they were not alive after the birth, maybe the young female being nervous very much did not nursed the new born cubs.

Best regards, Liba Veselá

Appendix 4

Full list of zoos in the European Amur leopard captive breeding programme.

Zoo	Country
Abakan	Russia
Agrate	Italy
Amiens	France
Augsburg	Germany
Aywaille	Belgium
Bekesbourne	UK
Berlin TP	Germany
Berlin Zoo	Germany
Burford	UK
Bussoleng	Italy
Chard	UK
Copenhagen	Denmark
Dortmund	Germany
Frankfurt	Germany
Helsinki	Finland
Hodenhagn	Germany
Kaunas	Lithuania
Kazan	Russia
Khabarovs	Russia
Khar Kov	Ukraine
Leipzeig	Germany
Lyons	France
Marwell	UK
Montpellier	France
Moscow	Russia
Mulhouse	France
Mulhouse	Germany
Nikolaev	Ukraine
Novosibirsk	Russia
Olomouc	Czeh Republic
Ostrava	Czeh Republic
Prague	Czeh Republic
Rostov	Russia
Rotterdam	Netherlands
Seversk	Russia
Sofia	Bulgaria
St Petersburg	Russia
Stralsund	Germany
Szeged	Hungary
Tallinn	Estonia
Twycross	UK
Yarmouth	UK
Zagreb	Croatia
Zurich	Switzerland

Appendix 5

Electronic copy of the database structure in Microsoft Access (compatibility mode).