A survey on zoo mortality over a 12-year period in Italy

Frine Eleonora Scaglione [Corresp.,] [1] , **Angelica Ferro** [1] , **Paola Pregel** [1] , **Enrica Berio** [1] , **Francesca Tiziana Cannizzo** [1] , **Bartolomeo Biolatti** [1] , **Enrico Bollo** [1]

[1] Department of Veterinary Science, University of Turin, Grugliasco, Italy

Corresponding Author: Frine Eleonora Scaglione
Email address: frineeleonora.scaglione@unito.it

Background. The zoo is a unique environment where to study animals. Zoos have a long history of research into aspects of animal biology, even if this was not the primary purpose for which they were established. The data collected from zoo animals can have a great biological relevance and they can tell us more about what these animals are like outside the captive environment. In order to pledge the health in all captive animals, it is important to perform a post-mortem examination in all animals that die in the collection. **Methods.**The mortality causes of two hundred eighty two mammals dead between 2004 and 2015 in three different Italian zoos (a Biopark, a Safari Park and a private conservation center) have been investigated. **Results.** Post mortem findings have been evaluated reporting the cause of death, zoo type, year and animal category. The animals frequently died for infectious diseases, in particular in ruminants causes of death were mostly related to gastro-intestinal pathologies. Also pulmonary diseases were very common in each of the considered zoo. Moreover, death causes were attributable to traumas, due to fighting with conspecifics or during mating. Genetic diseases and also malformations have been registered. **Discussion.** This research was a confirmation of how conservation, histology and pathology are all connected through individual animals. These application fields are extremely important to maintain populations of rare and endangered species and to know more about their morphological and physiological conditions. They are also useful to control pathologies, parasites and illnesses that can have a great impact on those captive species. Finally, this study underlines the importance of a close collaboration among veterinarians, zoo biologists and pathologists. Necropsy findings can help conservationists to determine how to support wild animal populations.

A survey on zoo mortality over a 12-year period in Italy

Frine Eleonora Scaglione *, Angelica Ferro *, Paola Pregel *, Enrica Berio *, Francesca Tiziana Cannizzo *, Bartolomeo Biolatti *, Enrico Bollo *

*University of Turin, Department of Veterinary Science, Largo P. Braccini 2, 10095 Grugliasco (To), Italy

Correspondingauthor:

Frine Eleonora Scaglione

e-mail: frineeleonora.scaglione@unito.it

Abstract

Background The zoo is a unique environment where to study animals. Zoos have a long history of research into aspects of animal biology, even if this was not the primary purpose for which they were established. The data collected from zoo animals can have a great biological relevance and they can tell us more about what these animals are like outside the captive environment. In order to pledge the health in all captive animals, it is important to perform a post-mortem examination in all animals that die in the collection.

Methods. The mortality causes of two hundred eighty two mammals dead between 2004 and 2015 in three different Italian zoos (a Biopark, a Safari Park and a private conservation center) have been investigated.

Results. Post mortem findings have been evaluated reporting the cause of death, zoo type, year and animal category. The animals frequently died for infectious diseases, in particular in ruminants causes of death weremostly related to gastro-intestinal pathologies. Also pulmonary diseases were very common in each of the considered zoo. Moreover, death causes were attributable to traumas, due to fighting with conspecifics or during mating. Genetic diseases and also malformations have been registered.

DiscussionThis research was a confirmation of how conservation, histology and pathology are all connected through individual animals. These application fields are extremely important to maintain populations of rare and endangered species and to know more about their morphological and

physiological conditions. They are also useful to control pathologies, parasites and illnesses that can have a great impact on those captive species.

Finally, this study underlines the importance of a close collaboration among veterinarians, zoo biologists and pathologists.

Necropsy findings can help conservationists to determine how to support wild animal populations.

INTRODUCTION

Zoos have always been considered as establishments where wild animals are kept for exhibition (other than a circus or a pet shop) to which members of the public have access, with or without charge for admission, for a minimum period of seven calendar days per year (Hoseyet al., 2009). Many zoos around the world keep animals confined to small spaces compared to their wide-ranging peers in the wild. Due to spatial constraints the captive environments have difficulty in providing the ideal setting for their natural behaviour, like hunting, resulting in welfare issues among captive animals (Morgan and Tromborg, 2007). Sometimes, animals in captivity exhibit abnormal behaviour such stereotypes (Vaz et al., 2017) or aggressiveness (Salas et al., 2016) due to poor welfare, since behaviour is an animal's "first line of defence" in response to environmental change, i.e., what animals do to interact with, respond to, and control their environment (Mench, 1998). Moreover, in literature, the pathologies affecting captive animals have been showed to be different from the ones affecting wild populations (Seeley et al., 2016; Strong et al., 2016).

Fortunately nowadays, the concept of zoo has changed. Many associations cooperate together to give a new point of view about zoos. It is important to highlight that zoos are not simple cages where animals are trapped, as believed by many people. They should be considered for their aims and goals. One of the key goals of many captive management programs is the eventual reintroduction of species back

into the wild. Zoos exhibit species to educate the public and cultivate its appreciation of conservation or research programs. Zoos offer their visitors "edu-trainment" through shows, contact areas, and interactive exhibits. They also begin to reflect on their reason of being, along with issues related to animal welfare, such as behavior, exhibit design, and nutrition (Griffin et al., 1992).

There are many types of modern zoos, like safari parks, conservation centers, landscape immersions, ecosystem exhibits, and also bioparks and sustainable zoos. Research, education and conservation are functions which, in the last one hundred years or so, have been grafted onto the recreational rootstock of zoos (Robinson, 1989).

Keeping wild animals in captivity has advantages, first of all, for animals (conservation can be viewed as beneficial for populations of animals, if not always for individual animals kept in captivity) and for humans as well (education, conservation, recreation and scientific discovery). Wild animals in captivity may not necessarily experience negative welfare and may, in some cases, be better than they would be in the wild (Bostock, 1993).

Conservation of endangered species is now one of the major goals of accredited zoos. The emphasis on a conservation role for zoos grew greatly in importance during the 1970s and 1980s, prompted partly by the zoos themselves and partly by external pressures, such as new international treaties and national legislation (Hosey et al., 2009).Another important aspect related to conservation is biodiversity.

Today, the term "conservation" and "biodiversity" are often used together, to make explicit the distinction between the conservation of living organism and non-living structures, such as buildings or books (Hosey et al., 2009). Another way of defining biodiversity would be as, the totally of genes, species and ecosystem in a region"(WRI/IUCN/UNEP/FAO/UNESCO, 1992). The role of the zoo in the conservation of biodiversity can be defined in four general areas:

- maintenance of captive stocks of endangered species; this is the idea of zoo that can act as a kind of 'ark';
- support for, and practical involvement with, in situ conservation projects. Zoos could contribute to this with, amongst other things, animal planning expertise, infrastructure, and financial support;
- education and campaigning about conservation issues; this can be achieved through enclosure design, signage, keeper talks, interactive education, animal shows... Indeed, it is as important sometimes to keep species of low conservation importance in zoos as it is to keep the high-priority species, because they may be more useful in promoting the conservation message by enhancing people's zoo experience of animals.
- research that benefits the science and practice of conservation; for many years, research conducted on zoo animals tended to be concerned primarily with anatomy and taxonomy, but there is a huge potential in zoo to undertake behavioral, genetic, and physiological research that contributes to the *in situ* and *ex situ* conservation of endangered species (Ryder and Feistner, 1995).

These roles and activities have been pointed out in three documents: "The World Zoo Conservation Strategy" (IUDZG/CBSG, 1993), "TheWorld Zoo and Aquarium Conservation Strategy" (WAZA, 2005) and "Turning the Tide" (Hosey et al., 2009; WAZA, 2009).

The zoo is a unique environment where to study animals. Unlike in the wild, the animals are easily accessible to the researcher, so from zoo, in the framework of a structured search and with correct licenses, animals data can be collected that would be logistically very difficult to get from their wild counterparts. Once more, unlike in the wild, some manipulations may be possible in the zoo to take

106 research beyond purely observational and into experimental approaches (Hosey et al., 2009), even if
107 some data can be biased by captivity (i.e. behavior, hunting….).
108 Zoos have a long history of research into aspects of animal biology, even if this was not the primary
109 purpose for which they were established (Hutchins, 2001).
110 The data collected from zoo animals can have a greater biological relevance than the data obtained
111 from the laboratory, and they can tell us more about what these animals are like outside the captive
112 environment (Hosey et al., 2009).
113 Therefore, many zoos undertake their research in collaboration with each other and with other
114 agencies, such as universities and conservation agencies. Indeed, universities and zoos can
115 complement each other, for example in topics such as the control and analysis of behavior,
116 conservation of endangered species, and education of students and the general public (Fernandez and
117 Timberlake, 2008). One of the greatest examples about the importance of research in zoo animals is
118 the discovery and management of diseases.
119 Diseases may be 'of concern' to zoos either because of the risk of direct losses of animals, or because
120 of the impact on the zoo of required measures in the case of an outbreak.
121 Each zoo will have different 'diseases of concern', depending on its geographical location and the
122 types of animal in its collection, which may vary quite widely from collection to collection, and over
123 time.
124 Diseases can be considered under four broad headings for all zoos:
125 - infectious diseases;
126 - degenerative diseases;
127 - genetic diseases;
128 - nutritional diseases (Hoseyet al., 2009).

Also capture, restraint, and anesthesia are stressful procedures for animals, and particularly so for wild species. It may be better to leave an animal with superficial injury to heal on its own without treatment if the only alternative is capture and full anesthesia. Veterinary treatment may have adverse effects on an animal's reproductive status, or may result in aggression from conspecifics when an individual is removed for treatment and then returned into a social group. Medication that can be administered in food or drinking water may be an option when capture and injection of drug is not desirable from a welfare perspective, or when it would put veterinary staff or keepers at high risk of injury. Euthanasia is also an option (Hoseyet al., 2009).

Preventive medicine and care play a very important role in zoos. The preventive medicine program for captive wild animals includes: stock selection, quarantine, routine health monitoring and maintenance, enclosure design, pest control, sanitation, and an employee health program. The overall goals of a preventive medicine program are to prevent disease from entering the animal collection, to assure that the animals are properly maintained, and to avoid dissemination of diseases to other institutions, or to free-ranging populations if collection animals belong to a reintroduction program (Norton, 1993).

Preventive medicine often starts with the careful selection of new animals and a period of quarantine or isolation.

In order to pledge the health in all captive animals, it is important to perform a post-mortem examination in all animals that die in the collection and also on wild and feral animals found dead on the zoo grounds (Hosey et al., 2009). Many Species Survival Plans (SSPs) have extensive necropsy protocols, so the appropriate SSP Veterinary Advisor should be consulted in advance for this information (Silberman, 1988).

Proper disposal of animal carcasses is essential for both human and animal health, as well as to comply with local and federal regulations (Hinshaw et al., 1996).

Long-term post-mortem records provide useful data on trends in health, both for individual zoos and among the wider zoo community, and this information can then help future decisions about health care in living animals.

The aim of the study was to evaluate the mortality causes to highlight the importance of post-mortem examination and its role in the preventive medicine and, secondly, to consider the importance of the veterinarian collaboration and cooperation into zoological gardens.

There are potential criticisms in this paper. Due to privacy policies, there is a lack of data about the animal inventory in relation to the number of necropsies. The authors are not allowed to report the data regarding the number of new animals arriving in the zoo, the number of births, the number of animals sent to other zoos, and this all influences the number of dead animals.

MATERIALS AND METHODS

Sample Collection

The study on the causes of death in zoo animals was performed taking into account the years between 2004 and 2015. It was decided to focus on the Order of mammalians only, which has been divided into four categories: monogastric herbivores, ruminants, carnivores and omnivores. Two hundred eighty twonecropsies have been carried out.

The animals, coming from three different Italian zoos (a Biopark, a Safari Park and a private conservation center), were referred to the Department of Veterinary Science of the University of Turin (Italy).

Sample analysis

Necropsy examination was performed for each animal by two pathologists. A file was filled in with the following fields: assigned number, autopsy date, zoo of origin, species, sex, age, sampled organs.

Gross examinations were performed for each animal. Based on the macroscopic findings, the pathologists sampled organs for the histological and/or microbiological investigations.

The organs were fixed in 10% neutral buffered formalin for histological examination. The samples were paraffin-embedded and sections of 4 μm were stained with hematoxylin and eosin. Histochemical or immunohistochemical staining were performed, if necessary. All the possible differential diagnoses were taken into account. Bacteriological, virological and parasitological investigations were performed, if needed.

Macroscopical and/or microscopic findings were classified according to the cause of death, including spontaneous pathology, infectious, genetic, complications (e.g. anesthesiological and surgical problems, management …) and other causes (e.g.: degenerative, neoplasia, nutritional and not determined diseases).

Statistical Analysis

The obtained data were analyzed by GraphPad Prism (vers. 6.0; GraphPad Software, California, USA). The association between the different tested variables was assessed by $\chi 2$ Test. All the results were considered statistically significant with the value $p< 0.05$.

RESULTS

In Table 1 and Figure 1, the total number of dead animals and their causes of death in the three different zoos were summarized.

Animals were classified according to their digestive system, with reference to the three zoos. Out of the 282 dead animals, 45 were monogastric herbivores, 175were ruminants, 54carnivores, and 8of them were omnivores.

196 A statistically significant association (P<0.01) between the zoo and the category of animals was
197 detected.
198 In Table 2, animals were analyzed separately according to the provenience from the different zoos, and
199 they were classifiedon the basis of their digestive system and to the cause of death. A statistically
200 significant association has been revealed between thecategory of dead animalsand the three zoos
201 (p<0.0001).Moreover, when the zoos were considered together, a statistically significant association
202 has been revealed also between the category of dead animals and the cause of death (p<0.0001; Fig.2).
203 In Zoo 1 out of the 60 dead animals, 25 (41.7%) were monogastricherbivores and 19 (76%) of them
204 died for infectious diseases. Out of the 31 (51.7%) ruminants, 22(71%) died for infectious diseases. In
205 Zoo 2, out of the 162 dead animals, 105 (64.8%) were ruminants, and 75 (71.4%) died for infectious
206 diseases, as well as 14 (29.2%) of the 48 (29.6%) carnivores. Fifteen (31.2%) carnivores died for
207 genetic diseases or malformations and 5 (10.4%) for complications. In Zoo 3, out of the 60 dead
208 animals, 30 (76.9%) out of the 39 (65%) ruminants and 11 (73.3%) out of the 15 (25%) monogastric
209 herbivoresc died for infectious diseases.
210 In Zoo 1, the highest mortality was revealed in 2013, when 15animals died (25%) and out of them,
211 12(80%) for infectious diseases (Table 3).
212 In 2015, 12 deaths were registered (20%) and out of them 10 (83.3%) for infectious diseases. Out of
213 the 15 animals which died in 2013 in Zoo 1, 7 (46.7%) were monogastricherbivores and 7 (46.7%)
214 were ruminants (Table 4).
215 In 2015, out of the 12 deaths registered, 5 (41.7%) were represented by monogastricherbivores and 7
216 (58.3%) by ruminants. In Zoo 2 mortality was particularly high in 2009, with 32 (19.7%) deaths, 25of
217 which (78.1%) for infectious disease (Table 5).

The most significant years for mortality in Zoo 2 were from 2006to 2010, and involved mostly carnivores and ruminants (Table 6).

The highest mortality in Zoo 3 was present in 2004, with 39 (65%) deaths (Table 7).

Among them, 29(74.3%) died for infectious disease. In 2005 19(31.7%) deaths were registered and 12(63.1%) of them were attributable to infectious diseases.

In Zoo 3 in 2004, out of the 39 (65%) dead animals, 29 (74.3%) were ruminants and 7 (17.9%) were monogastric herbivores (Table 8). In 2005, out of 19 (31.7%) dead animals 10 (52.6%) were ruminants, 7 (36.8%) were monogastric herbivores, and 2 (10.5%) carnivores (Table 8).

Neoplasia, degenerative, nutritional and not determined diseases were classified as “other” in all the zoos, since some pathologies were not clearlyascribable to a specific cause (e.g.: when hepatic failure occurred consequently to steatosisthe primary cause of this disease could be attributable both to degenerative or a nutritional factor)

Post-mortem Findings in Zoos

The results obtained from laboratory investigations performed on animal death in the three zoos are reported in Tables 9-11.

DISCUSSION

After the death of an animal, zoos are always advised to perform post-mortem examinations. The responsibility for this decision normally lies with the zoo veterinarian. Fast retrieval storage , and disposal of the carcass, contact with a specialized pathologist and record keeping are good practices to facilitate high quality of post-mortem examinations. The safety of the staff in contact with dead

240 animals is also relevant for inclusion in the protocol for post-mortem procedures (EU. Zoo Directive.
241 2015).
242 The cause of death for each animal dying in the collection needs to be established where reasonable
243 and practicable to do so, including, the majority of cases, the examination of the specimen by a
244 veterinary surgeon, pathologist or practitioner with relevant experience and training (EAZA,
245 2014).Often parasites, nutritional deficiencies, or dental disease, may be present in the animal
246 collection without causing any obvious symptoms or clinical signs. Their detection at post-mortem
247 examination frequently indicates that diagnostic tests or treatments should be performed on the
248 remaining animals before clinical symptoms or disease transmission occur (Defra, 2012).
249 In this survey a general analysis has been reported, conducted by a group of veterinary pathologists, on
250 the most common causes of death in zoo animals, over a twelve-year period. In order to provide
251 complete and satisfactory data, 282necropsies of zoo animals were performed.
252 In the study three different types of zoo (a Biopark, a Safari Park and a private conservation center)
253 were included, since each one of these zoos had a different approach to the idea of animals' keeping, as
254 described in the introduction.
255 Interesting considerations can be made, on the basis of the obtained results.
256 Depending on the type of zoo, dead animals' categories and causes of death were differently
257 represented, probably due to the different management and enclosures applied.
258 Trauma can occur as a result of poor enclosure design or during capture and transport. Moreover,
259 animals may also be injured in fights with conspecifics, particularly after introduction into new social
260 group, or during mating. In fact forty seven animals (16.7%) of the study died for trauma due to
261 injuries by conspecifics or capture.

Zoo animals are protected from some health risks that are normally faced by wild animals, thanks to measures such as vaccination (Fernández-Bellon et al., 2017)and the provision of an adequate diet. At the same time, contracting an illness remains an inevitable part of zoo animal life. In fact, diseases may be spread to zoo animals through contact with conspecifics, free-ranging species, pests, such as rats and mice, keepers or visitors (Schaftenaar, 2002; Zhang et al., 2017). The study highlights that the main cause of death on captive mammals, was attributed to infectious disease (177animals, 62.8%). Similar data were reported for each of the examined zoos and the 71.7% of the examined animals dead due to infective agents were ruminants.

According to scientific literature; the ruminants frequently die for infectious diseases, mostly related to their intestinal flora swing.

Links between the diets fed and gastrointestinal problems have been reported (Zenker et al., 2009; Schilcher et al., 2013; Taylor et al., 2013). Moreover, diet and lack of structured feed items can be associated with acidosis in ruminants (Gattiker 2014).

Not only enteritis and other pathological conditions of the digestive system have been pointed out, but also pulmonary diseases. In fact, in every zoo (as described in Tables 9, 10 and 11), pneumonia and other pulmonary diseases were very common.

Respiratory infections are multifactorial diseases (Jubb et al., 2015).Climate change can probably be one of the factors which can increase the occurrence, distribution and prevalence of infectious diseases of the lung (Mirsaeidi et al., 2015). This result is also in agreement with the literature, in particular for livestock. Different factors could affect livestock diseases when influenced by climate changes, such as the virulence of the pathogen itself, presence of vectors (if any), farming practices and land use, zoological and environmental factors and the establishment of new microenvironments and microclimates. The interaction of these factors is an important consideration in forecasting how

livestock diseases may be diffused. Risk assessments should focus on looking for combinations of factors that may be directly influenced by climate changes, or that may be indirectly affected through changes in human activity, such as transport and movement of animals, intensity of livestock farming and habitat change (Gale et al., 2009).

In this study we also considered the mortality rate for each year. These data confirm that, even if there are no trigger factors of an uncontrollable epidemic in a territory, a different animal species in different years may be more prone to death.

Moreover, as demonstrated in this study, and also reported in a previous paper (Scaglione et al., 2010), in white lion cubs an increased risk of inbreeding and genetic abnormalities can be a peculiar element in zoos that are involved in the breeding of rare or endangered species, when genetic diversity can be low in captive populations (Hoseyet al., 2009).

In Zoo 2, out of 48 dead carnivores, 14 (29.2%) died for infectious diseases and 15 (31.2%) died for genetic diseases or malformations. These latest findings, due to inbreeding, arose in felines, and in particular in the cubs. As described in the introduction, the use of studbooks may limit the inbreeding and genetic abnormalities occuring in zoo animals (Leipold, 1980).

In literature different studies have been conducted on animals' necropsies and they normally focus on single animal's species(EAZWV; 2008; Joyce-Zuniga et al., 2014).

A holistic approach was carried out in 1983, from San Diego Zoo and the Department of Pathology of Zoo Animals, which conducted a survey on zoo animal necropsies over a fourteen-year period (Griner, 1983). Necropsies of wildlife and zoo animals were performed, taking into account all the species and all the taxa. The veterinarians highlighted the importance of necropsies and collection of data.

CONCLUSIONS

In conclusion, this research has been carried out to underline how conservation, histology and pathology are:

1. all connected through individual animals.
2. extremely important to maintain populations of rare and endangered species and to know more about their morphological and physiological conditions.
3. useful to control diseases, parasites and illnesses that can have a great impact on those captive species.

Finally, this study underlines the importance of:

1. a close collaboration among veterinarians, zoo biologists and veterinary pathologists.
2. necropsy findings that can help to determine how to support wild animal populations.

330

331

332

333

334 References

335

336 Bostock SC. 1993. *Zoos and Animal Rights*, London, UK: Routledge. 227 p.

337 Defra (Departement for Enviroment, Food and Rural Affairs). 2012. *Secretary of State's Standards of*

338 *Modern Zoo Practise*.34-62.

339 EAZA (European Association of Zoos and Aquaria). 2014. *Standards for the Accomodation and Care*

340 *of Animals in Zoos and Aquaria*. 21 p.

341 EAZWV (European Association Zoo and Wildlife Animals Veterinarian). 2008.*Evaluation of Okapi*

342 *(Okapiajohnstoni) Necropsy Reports and Studbook Data as part of the EAZWV Summer School.*

343 Leipzig, Germany.

344 EU Zoo Directive. 2015. *Good Practices Document*. Luxembourg, Publications Office of the European

345 Union. 192 p.

346 Fernandez EJ, Timberlake W. 2008. Mutual benefits of research collaborations between zoos and

347 academic institution. *Zoo Biology* 27: 470-487.

348 Fernández-Bellon H, Vergara-Alert J, Almagro V, Rivas R, Majó N, Busquets N, Ramis A.

349 2017. Evidence that avian influenza vaccination induces long-lived immune responses in zoo

350 birds.*Veterinary Records*180(22):544.

Gale P, Drew T, Phipps LP, David G, Wooldridge M. 2009. The effect of climate change on the occurrence and prevalence of livestock diseases in Great Britain: A review. *AppliedMicrobiologyBiotechnology*5: 1409-1423.

Gattiker C, Espie I, Kotze A, Lane EP, Codron D, Clauss M. 2014. Diet and diet-related disorders in captive ruminants at the national zoological gardens of South Africa. *Zoo Biology* 33(5):426-432.

Griffin DR. 1992. *Animal Minds*, Chicago, IL; University of Chicago Press. 376 p.

Griner LA. 1983. *Pathology of Zoo Animals: A Review of Necropsies Conducted over a Fourteen-Year Period at the San DiegoWild Animal Park*. Zoological Society of San Diego, San Diego

Hinshaw KC, Amand WB, Tinkelman CL. 1996. Preventive medicine. In: Kleiman DG, Allen ME, Thompson KV, Lumpkin S. ed. *Wild Mammals in Captivity: Principles and Technique*. 1st ed. Chicago, IL: University of Chicago Press p.16-24.

Hosey GR, Melfi V, Pankhurst S. 2009. *Zoo Animals: Behaviour, Management, and Welfare*. 2nd ed. Oxford, UK: Oxford University Press. 688 p.

Hutchins M. 2001. Research: overview. In: Bell CE, ed. *Encyclopedia of the World's Zoos. 1st ed.* Chicago, IL: Fitzroy Dearborn p. 1076-1080.

IUDZG/CBSG (International Union of Directors of Zoological Gardens/ Conservation Breeding Specialist Group), 1993. *Executive Summary, The World Zoo Conservation Strategy; The Role of the Zoos and Aquaria of the World in Global Conservation*. Chicago Zoological Society, Brookfield, Illinois, U.S.A. 14p.

Joyce-Zuniga NM, Roesler J, Andrus CH, Sutherland-Smith M, Rideout BA, Pye GW. 2014. Gastrointestinal torsions and intussusceptions in northern koalas (*Phascolarctoscinereus*) at San Diego Zoo (1976-2012). *Journal of Zoo and Wildlife Medicine* 45: 118-126.

374 Jubb KVE, Kennedy PC, Palmer NC. 2015.Ed. By M. Grant Maxi. *Jubb, Kennedy and Palmer's*
375 *Pathology of Domestic Animals*.6thEdition

376 Leipold HW. 1980. Congenital defects of zoo and wild mammals: a review. In: Montali RJ, Migaki G,
377 ed. *The Comparative Pathology of Zoo Animals*. Washington, Washington DC: Smithsonian
378 Institutionp. 457-470.

379 Mench JA. 1998. Why it is important to understand animal behavior. *ILAR Journal* 39: 20–26.

380 Mirsaeidi M, Motahari H, TaghizadehKhamesi M, Sharifi A, Campos M, Schraufnagel DE.
381 2016. Climate Change and Respiratory Infections. *Annals of the American Thoracic*
382 *Society*13(8):1223-1230.

383 Morgan KN, Tromborg CT. 2007. Sources of stress in captivity*Applied Animal Behaviour*
384 *Science* 102: 262–302.

385 Norton TM. 1993. Preventive medicine protocols for reintroduction programs. In: *Proceeding of the*
386 *Annual Meeting of the American Association of Zoo Veterinarians*. Philadelphia, USA: American
387 Association of Zoo Veterinarians p. 323-332.

388 Robinson MH. 1989. The zoo that is not: education for conservation. *ConservationBiology*3: 213-215.

389 Ryder OA, Feistner ATC. 1995. Research in zoos; a growth area in conservation. *Biodiversity and*
390 *Conservation*, 4: 671-677

391 Salas M, Temple D, Abáigar T, Cuadrado M, Delclaux M, Enseñat C, Almagro V, Martínez-
392 Nevado E, Quevedo MÁ, Carbajal A, Tallo-Parra O, Sabés-Alsina M, Amat M, Lopez-Bejar M,
393 Fernández-Bellon H, Manteca X. 2016. Aggressive behavior and hair cortisol levels in captive
394 Dorcas gazelles (*Gazelladorcas*) as animal-based welfare indicators. *Zoo Biology* 35(6):467-473.

Scaglione FE, Schröder C, Degiorgi G, Zeira O, Bollo E. 2010. Cranial malformations in related white lions (*Pantheraleokrugeri*). *Veterinary Pathology* 47: 1095-1099.

Schaftenaar W. 2002. Use of **vaccination** against foot and mouth disease in **zoo** animals, endangered species and exceptionally valuable animals.. *Revue Scientifique Et Technique*. 21(3):613-623.

Schilcher B, Baumgartner K, Geyer H, Liesegang A. 2013. Investigations on rumen health of different wild ruminants in relation to feeding management. *Journal of zoo and aquarium research* 1:28–30.

Seeley KE, Garner MM, Waddell WT, Wolf KN. 2016. A survey of diseases in captive red wolves (*Canisrufus*), 1997–2012. *Journal of Zoo and Wildlife Medicine* 47:83-90

Silberman MS. 1988. Compendium of Occupational Health & Safety Programs (Laboratory & Zoological Institutions). R.W. Woodruff Health Sci. Ctr. Emory Univ. Atlanta, GA. Guidelines of Zoo and Aquarium veterinary medical programs and veterinary hospitals.

StrongVJ,GrindlayD,Redrobe S, Cobb M, White K. 2016. A systematic review of the literature relating to captive great ape morbidity and mortality *Journal of Zoo and Wildlife Medicine* 47(3):697-710.

Taylor LA, Schwitzer C, Owen-Smith N, Kreuzer M, Clauss M. 2013. Feeding practices for captive greater kudus (*Tragelaphusstrepsiceros*) in UK collections as compared to diets of free-ranging specimens. *Journal of zoo and aquarium research* 1:7–13.

Vaz J, Narayan EJ, Dileep Kumar R, Thenmozhi K, Thiyagesan K, Baskaran N. 2017. Prevalence and determinants of stereotypic behaviours and physiological stress among tigers and leopards in Indian zoos. *PLoS One* 12(4):e0174711.

WAZA (World Association of Zoos and Aquariums). 2005. *Building a Future for Wildlife: The World Zoo and Aquarium Conservation Strategy*, Liebelfeld-Berne.

WAZA (World Association of Zoos and Aquariums). 2009. *Turning the Tide: A Global Aquarium Strategy for Conservation and Sustainability*, Liebelfeld-Berne

WRI/IUCN/UNEP/FAO/UNESCO (World Resources Institute/The World Conservation Union/United Nations Environment Programme in consultation with the Food and Agriculture Organization and the United Nations Education, Scientific and Cultural organization) 1992. Global *Biodiversity Strategy: Guidelines for Action to Save, Study and Use Earth's Biotic Wealth Sustainably and Equitably*. Washington, Washington DC: WRI.

Zenker W, Clauss M, Huber J, Altenbrunner-Martinek B. 2009. Rumen pH and hoof health in two groups of captive wild ruminants. In: Clauss M, Fidgett A, Hatt J-M, Huisman T, Hummel J., Nijboer J., Plowman A. editors. Zoo animal nutrition IV. Fürth, Germany: FilanderVerlag: p 247–254.

Zhang H, Shan F, Zhou X, Li B, Zhai JQ, Zou SZ, Wu MF, Chen W, Zhai SL, Luo ML. 2017. Outbreak and genotyping of canine distemper virus in captive Siberian tigers and red pandas. Scientific Reports 7(1):8132.

Table 1(on next page)

Total number of dead animals for each zoo classified according to death causes

	Infect. diseases	Traumas	Complications	Genetic diseases and malformations	Other	Tot.
zoo 1	43	12	2	0	2	59
zoo 2	91	25	17	15	14	162
zoo 3	43	10	1	0	4	58
	177	47	20	15	20	279

1

Table 2(on next page)

Total number of dead animals for each zoo classified according to their digestive system and death causes

	Monogastric herbivores			Ruminants			Carnivores			Omnivores			TOTAL
	zoo 1	zoo 2	zoo 3	zoo 1	zoo 2	zoo 3	zoo 1	zoo 2	zoo 3	zoo 1	zoo 2	zoo 3	
Infect. diseases	19	1	11	22	75	30	1	14	2	1	1		177
Traumas	5	3	2	6	17	6	1	4	1		1	1	47
Complications		1		2	9	1		5			2		20
Genetic diseases and malformations								15					15
Other	1		2	1	4	2		10					20
Tot.	25	5	15	31	105	39	2	48	3	1	4	1	279

1

Table 3(on next page)

Total number of dead animals in Zoo 1 classified according to the year and cause of death

	Infect. diseases	Traumas	Complications	Genetic diseases and malformations	Other	Tot.
2005	1				1	2
2006	1	1	1			3
2007	2	2			1	5
2008	5					5
2009		1				1
2010	2	1				3
2011	2					2
2012	8	2				10
2013	12	2	1			15
2014		2				2
2015	10	1			1	12
tot	43	12	2	0	3	60

1

Table 4(on next page)

Total number of dead animals in Zoo 1 classified according to the year and animals' digestive system

	Monogastric herbivores	Ruminants	Carnivores	Omnivores	TOTAL
2005	1			1	2
2006		3			3
2007	2	3			5
2008	3	2			5
2009			1		1
2010	1	2			3
2011		2			2
2012	6	3		1	10
2013	7	7	1		15
2014		2			2
2015	5	7			12
	25	31	2	2	60

1

Table 5(on next page)

Total number of dead animals in Zoo 2 classified by animal category and cause of death

	Infect. diseases	Traumas	Complications	Genetic diseases and malformations	Other	Tot.
2004	13	1	3		2	19
2005	3	1	2		4	10
2006	7	4	1	7	1	20
2007	6	5	3		1	15
2008	7	3	5	6	1	22
2009	25	4	1	1	1	32
2010	13	3	1	1		18
2011	8	2	1		3	14
2012	7	2			1	10
2013	1					1
2014	1					1
tot	91	25	17	15	14	162

1

Table 6(on next page)

Total number of dead animals in Zoo 2 classified according to the year and animals' digestive system

	Monogastric herbivores	Ruminants	Carnivores	Omnivores	TOTAL
2004		15	3	1	19
2005		5	5		10
2006		8	11	1	20
2007	2	6	6	1	15
2008	1	9	11	1	22
2009	2	26	4		32
2010		16	2		18
2011		10	4		14
2012		9	1		10
2013			1		1
2014		1			1
tot	5	105	48	4	162

1

Table 7 (on next page)

Total number of dead animals in Zoo 3 classified by animal category and cause of death

	Infect. diseases	Traumas	Complications	Genetic diseases and malformations	Other	Tot.
2004	29	5	1		4	39
2005	12	5			2	19
2006	2					2
tot	43	10	1	0	6	60

1

Table 8(on next page)

Total number of dead animals in Zoo 3 classified according to the year and animals' digestive system

	Monogastric herbivores	Ruminants	Carnivores	Omnivores	TOTAL
2004	7	29	1	2	39
2005	7	10	2		19
2006	1		1		2
2007					0
2008					0
2009					0
2010					0
2011					0
2012					0
tot	15	39	4	2	60

1

Table 9(on next page)

Post-mortem findings in Zoo 1

Registernumber	Year	Specie	Causes of death	Lab. findings
1A	2005	Horse	Septicemia	Cl. Perfrigenstype D
2A	2005	Skunk	Pulmonaryemphysema	-
3A	2006	Fallowdeer	Trauma	-
4A	2006	Fallowdeer	Toxemiasyndrome	-
5A	2006	Ilama	Pneumonia	-
6A	2007	Goat	Ab ingestis pneumonia	-
7A	2007	Grey squirrel	Trauma	-
8A	2007	Deer	Trauma	-
9A	2007	Goat	Pneumonia	
10A	2007	Patagonia hare	Septicemia	Pseudotubercolosis
11A	2008	Ilama	Pneumonia	-
12A	2008	Ilama	Pneumonia	-
15 a	2008	Patagonia hare	Septicemia	-
13A – 14A	2008	Domestic rabbits	Pneumonia	-
16A	2009	Siberiantiger	Internalhemorrhage	-
17A	**2010**	**Tibetan goat**	**ClostridialEnterocolitis**	**Clostridium**
18A	2010	Hare	Trauma	
19A	2010	Tibetangoat	Septicemia	E.Coli
20A	2011	Ilama	Septicemia	Salmonellosis
21A	2011	Antelope	Pleuritis	-
22A	2012	Antelope	Septicemia	-
23A	2012	Deer	Cranial trauma	-
24A	2012	Deer	Septicemia	Actinobacillosis
25A	2012	Hare	Trauma	-
26A	2012	Swine	Pericarditis	-
27-31A	2012	Hares	Pneumonia	-
32A	2013	Deer	Septicemia	Enterococcus
33A	2013	Ilamacalf	Pneumonia	-
34-35A	2013	Eulemurs	Trauma	-
36A	2013	Hare	Septicemia	Pasteurella multocida

37-40A	2013	Rabbits	Pneumonia	-
41A	2013	Siberiantiger	Pulmonaryhemorrhage	-
42-43A	2013	Mohrgazelles	Pneumonia	-
44A	2013	Thompson gazelle	Distocya	-
45-46A	2013	Deer	Pneumonia	-
47-48A	2014	Mohrgazelle	Trauma	-
49A	2015	Horse	Liverfailure	-
50-51A	2015	Thompson gazelle	Septicemia	-
52A	2015	Watusi	Enteritis	-
53A	2015	Gazelle	Pneumonia	-
54A	2015	Yak	Pneumonia	-
55A	2015	Goat	Trauma	-
56A	2015	Goat	Pneumonia	-
57-60A	2015	Rabbit	Pneumonia	

1

Table 10(on next page)

Post-mortem findings in Zoo 2

Data	Years	Species	Causes of death	Lab. findings
1B	2004	Lion	Neoplasia	Alveolar Carcinoma
2B	2004	Opossum	Encephalitis	-
3B	2004	Goat	Pneumonia	-
4B	2004	Dromedary	Enteritis	-
5B	2004	Antelope	Blood poisoning	-
6B	2004	Goat	Pneumonia	-
7B	2004	Antelope	Pneumonia	-
8B	2004	Yak	Clostridiosis	*Clostridium* spp. *E.coli*
9B	2004	Ilama	Thoracic Trauma	-
10B	2004	Nilgai	Clostridiosis	*Clostridium perfringens*
11B	2004	Watusi	Chronic gastritis and entheritis	-
12B	2004	Dromedary	Septic Granuloma	*Thricostrongylus* spp Protostrongylus spp. Nematodirus spp.
13B	2004	Blesbuck	Pneumonia and pleuritis	*Thricostrongylus* spp. Protostrongylus spp. Ostertagia spp.
14B	2004	Eland	Blood poisoning	-
15B	2004	Eland	Pneumonia	E.coli
16B	2004	Lion	Paraplegia (Euthanasia)	-
17B	2004	Blesbuck	Pneumonia and pleuritis	-
18B	2004	Goat	Pneumonia	
19B	2004	Lion	*Ab ingestis* Pneumonia	-
20B	2005	Giraffe	Heart attack	-
21B	2005	Goat	Not determined	-
22B	2005	Goat	Not determined	-
23B	2005	White Lion	*Ab ingestis* Pneumonia	-

24B	2005	Lion	Neonatal Mortality	-
25B	2005	Lion	Mesothelioma	-
26B	2005	White lion	Pneumonia	-
27B	2005	Antelope	Severe Pneumonia	-
28B	2005	Tiger	Peritonitis	-
29B	2005	Barbary sheep	Trauma	-
30B	2006	Tiger	Entheritis	-
31B	2006	Racoon	Trauma (Thoracic hemorrage)	-
32B	2006	Tiger	Not determined	-
33B	2006	White lion	Inborn malformation	-
34B	2006	Mouflon	Trauma	-
35B	2006	Lion	Maxillary hypoplasia	-
36B	2006	White Lion	Neonatalmortality	-
37B	2006	White Lion	Neonatalmortality	-
38B	2006	White Lion	Neonatalmortality	-
39B	2006	White Lion	Neonatalmortality	-
40B	2006	Waterbuck	Politrauma	-
41B	2006	Goat	Pneumonia	-
42B	2006	Waterbuck	Foreign body (Peritonitis)	-
43B	2006	Siberian Tiger	Severe pneumonia	-
44B	2006	Gemsbuck (Oryx)	Pneumonia	-
45B	2006	Waterbuck	Severe pneumonia	-
46B	2006	Eland	Trauma	-
47B	2006	White lion	Neonatal mortality	-
48B	2006	White lion	Severe pneumonia	-
49B	2007	Siberian Tiger	Severe pneumonia	-
50B	2007	Eland	Severe pneumonia	-
51B	2007	Racoon	Poisoning	-
52B	2007	Hippopotamus	Trauma	-
53B	2007	Wildebeest	Trauma	-
54B	2007	Dromedary	Abortion	*E.coli*

55B	2007	Gemsbuck (Oryx)	Trauma	-
56B	2007	Lion	Pneumonia	-
57B	2007	Tiger	Cranial trauma	-
58B	2007	Tiger	Suffocation	-
59B	2007	Tiger	Severe pneumonia	-
60B	2007	Siberian Tiger	Severe rhinitis and pneumonia	-
61B	2007	Gemsbuck (Oryx)	Infection	*Moraxella* spp.
62B	2007	Hippopotamus	Trauma	-
63B	2007	Buffalo	Blood poisoning	-
64B	2008	Lion	Trauma	-
65B	2008	Deer	Trauma	-
66B	2008	Tiger	Internal hemmorage	-
67B	2008	Baboon hamadryad	Ipothermia	-
68B	2008	Buffalo	Septicemia	-
69B	2008	White lion	Pneumonia	-
70B	2008	Waterbuck	Ipothermia	-
71B	2008	Gemsbuck (Oryx)	Septicemia	-
72	2008	White Lion	Neonatal mortality	-
73B	2008	White Lion	Neonatal mortality	-
74B	2008	White Lion	Neonatal mortality	-
75B	2008	Eland	Pneumonia	-
76B	2008	Barbary sheep	Trauma	-
77B	2008	Lion	*Ab ingestis* pneumonia	-
78B	2008	Lion	*Ab ingestis* pneumonia	-
79B	2008	Goat	Pneumonia	-
80B	2008	Patagonian hare	Enteritis	-
81B	2008	Lion	Neonatal mortality	-
82B	2008	Lion	Neonatal mortality	-
83B	2008	Lion	Neonatal mortality	-
84B	2008	Eland	Severe septicemia	-

85B	2008	Gemsbuck (Oryx)	Neonatal	-
86B	2009	Eland	Abdominal trauma	-
87B	2009	Waterbuck	Pneumonia	*E.coli*
88B	2009	Waterbuck	Trauma	-
89B	2009	Waterbuck	Enteritis	*E.coli*
90B	2009	Goat	Lymphoadenitis	-
91B	2009	Goat	Enteritis and pneumonia	*Staphylococcus xylosus; Streptococcus bovis; E.coli; Clostridium perfringens*
92B	2009	Goat	Enteritis	-
93B	2009	Waterbuck	Peritonitis	-
94B	2009	Waterbuck	Trauma	-
95B	2009	Waterbuck	Metritis	*E.coli; Streptococcus bovis*
96B	2009	Tiger	Pulmonary abscess	-
97B	2009	Tiger	Chronic nephritis	-
98B	2009	Barbary sheep	Enteritis	*Salmonella venezuelana*
99B	2009	Goat	Pneumonia	-
100B	2009	Hippopotamus	Trauma	-
101B	2009	Barbary sheep	Septicemia	-
102B	2009	Barbary sheep	Enteritis	-
103B	2009	Tibetan Goat	Enteritis	-
104B	2009	Barbary sheep	Enteritis	-
105B	2009	Barbary sheep	Enteritis	-
106B	2009	Ilama	Enteritis	*E.coli*
107B	2009	Dromedary	Abortion	-
108B	2009	Lion	Neonatal mortality	
109B	2009	Barbary sheep	Deterioration	-
110B	2009	White lion	Inborn disease (macroglossia)	-
111B	2009	Barbary sheep calf	Enteritis and pneumonia	-
112B	2009	Barbary sheep	Pneumonia	-

113B	2009	Barbary sheep	Enteritis	-
114B	2009	Goat	Pneumonia	-
115B	2009	White donkey	Colic	-
116B	2009	Wildebeest	Hemorragic peritonitis	-
117B	2009	Cameroon Goat	Abortion	-
118B	2010	Watusi	Pneumonia	-
119B	2010	Siberian tiger	Trauma	(diaphragmatic hernia)
120B	2010	Waterbuck	Pneumonia	-
121B	2010	Goat	Pulmonary congestion	-
122B	2010	Goat	Pulmonary congestion	-
123B	2010	Gemsbuck (Oryx)	Anesthesia	-
124B	2010	Sheep	Pulmonary congestion	-
125B	2010	Goat	Pericardial effusion	-
126**B**	2010	Gemsbuck (Oryx)	Parasitic hepatitis and pneumonia	-
127B	2010	Waterbuck calf	Neonatal mortality	-
128B	2010	Barbary sheep	Trauma	-
129B	2010	SIBERIAN TIGER	FALLOT PENTALOGY	-
130B	**2010**	**Antelope**	**Hepatitis**	**-**
131B	2010	Gemsbuck (Oryx)	Euthanasia	(septicemia)
132B	2010	Waterbuck	Trauma	-
133B	2010	Waterbuck	Septicemia	-
134B	2010	Waterbuck	Septicemia	-
135B	2010	Tibetan goat	Pericardial effusion	-
136B	2011	Siberian tiger	Euthanasia	-
137B	2011	Wildebeest calf	Mesenteric hemorrage	-
138B	2011	Dromedary	Neonatal mortality	-
139B	2011	Siberian tige	Trauma	-
140B	2011	Eland	Septicemia	-
141B	2011	Gesmbuck	Trauma and septicemia	-
142B	2011	Antelope	Not determined	-
143B	2011	Gemsbuck	Pneumonia	-
144B	2011	Siberian tiger	Abortion and septicemia	-
145B	2011	Dromedary	Pulmonary congestion and septicemia	-
146B	2011	Eland	Gastritis	-
147B	2006	Eland	Enteritis	-
148B	2011	Goat	Pulmonary edema	-
149B	2011	Tiger	Not determined	-

150B	2011	Antelope	Mycosis	-
151B	2012	Waterbuck	Septicemia	-
152B	2012	Waterbuck	Trauma	-
153B	2012	Giraffe	Septicemia	*Achromobacter xylosoxidans; Streptococcus bovis;Stenotrophomonas maltophila*
154B	2012	Cow	Septicemia	-
155B	2012	Bison	Enteritis	-
156B	2012	Cameroon goat	Enteritis	-
157B	2012	Goat	Trauma	-
158B	2012	Gemsbuck	Degradation	-
159B	2012	Goat	Pneumonia	-
160B	2012	Cheetah	Neoplasia	Pancreatic
161B	2013	Cheetah	Interstitial nephritis	-
162B	2014	Giraffe	Pericarditis	-

1

Table 11(on next page)

Post-mortem findings in Zoo 3

Registernumber	Years	Species	Causes of death	Lab. findings
1C	2004	Barbarysheep	Pulmunaryembolism	-
2C	2004	Ferret	Cirrhosis	-
3C	2004	Kangaroo	Pneumonia	-
4C	2004	TibetanGoat	Pneumonia	-
5C	2004	Cameroon sheep	Cysticercosis	TaeniaSaginata
6C	2004	TibetanGoat	Pneumonia	-
7C	2004	BarbarySheepcalf	Trauma	-
8C	2004	Ilama	Pneumonia and pericarditis	-
9C	2004	Kangaroo	Pneumonia	-
10C	2004	Kangaroo	Liverdisease	-
11C	2004	Kangaroo	Pneumonia	-
12C	2004	Crab-eatingMacaque	Liverfailure	-
13C	2004	Fallowdeer	Pneumonia	-
14C	2004	Fallowdeer	Pneumonia	-
15C	2004	Girgentana goat	Pneumonia	-
16C	2004	Blackbuck	Pneumonia	-
17C	2004	Fallowdeercalf	Trauma	-
18C	2004	Raccoon	Trauma	-
19C	2004	Barbarysheep	Pneumonia	-
20C	2004	Blackbuck	Pneumonia	-
21C	2004	Tibetangoat	Pneumonia	-
22C	2004	Barbarysheepcalf	Trauma	-
23C	2004	Tibetangoat	Pulmunary Edema	-
24C	2004	Goat	Pneumonia	-
25C	2004	Barbarysheep	Steatosis	-
26C	2004	Chital	Pneumonia	-
27C	2004	Barbarysheepcalf	Hemorrhagicenteritis	-
28-29C	2004	Barbarysheep	Pneumonia	-
30-32C	2004	Kangaroo	Pulmonary edema	-
33C	2004	Fallowdeer	Predation	-
34C	2004	Angora Goat	Septicemia	-
35C	2004	Blackbuck	Pneumonia	-
36C	2004	Barbarysheepcalf	Pneumonia	-
37-39C	2004	Tibetangoat	Pneumonia	-

40C	2005	Wallaby	Pulmonary edema	-
41C	2005	Wallaby	Septicemia	-
42C	2005	Squirrel	Trauma	-
43C	2005	Ferret	Trauma	-
44C	2005	Prairie dog	Hepatic neoplasia	-
45C	2005	Squirrel	Pneumonia	-
46C	2005	Ferret	Hemorrhagicenteritis	-
47C	2005	Antelope	Pneumonia	-
48C	2005	Barbarysheep	Trauma	-
49C	2005	TibetanGoat	Pneumonia and Pleuritis	-
50C	2005	Kangaroo	Pericardialeffusion and septicemia	-
51C	2005	Kangaroo	Steatosis	-
52C	2005	Barbarysheep	Pneumonia	-
53C	2005	Goat	Trauma	-
54C	2005	Angora goat	Pericardial effusion	
55C	2005	Fallowdeer	Pneumonia	-
56C	2005	Antelope	Peritonitis	-
57C	2005	Dwarfgoat	Trauma	-
58C	2005	Deer	Pneumonia	-
59C	2006	Blue monkey	Pulmonaryemphysema	-
60C	2006	Fox	Pneumonia	-

1

Figure 1

Number of dead animals for each zoo classified according to death causes

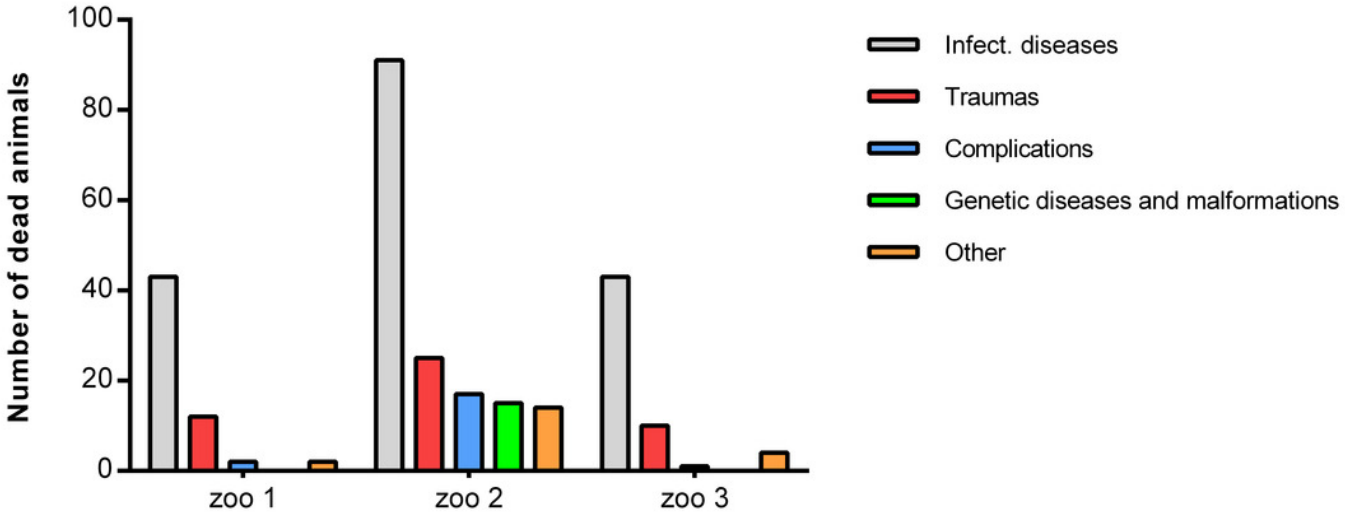

Figure 2

Number of dead animals classified according to their digestive system and death causes