Management protocols for animals in captive propagation and reintroduction programmes

S.K. MIKOTA and R.F. AGUILAR *

Summary: Species Survival Plans in North America — and comparable programmes in other parts of the world — address the management issues related to maintaining populations of endangered species in captivity. The need has been recognized for universal methods of assessing, evaluating, monitoring and preventing the transmission of disease to naive wildlife by reintroduced species. Standardized protocols are presented for evaluation of the health status of captive animals intended for release.


INTRODUCTION

Historically, infectious diseases have played an important role in captive wild animal management. The most obvious concerns have dealt with the possibility of epizootic or zoonotic diseases decimating a collection. Even an outbreak of non-lethal but debilitating disease might significantly affect the ability of a captive population to sustain itself. Most recently, however, the reintroduction of captive-bred animals into managed wild situations for repopulation has presented the problem of the potential dissemination of disease acquired in captivity to a naive wild population. Little is known about the effect of most infectious agents on wild animal populations. Some authors feel that the magnitude of the risk precludes the reintroduction of captive-bred wild animals (27, 33). Certain diseases known to affect domestic animals also affect wildlife species. The prevalence of many diseases in wild animals remains unknown, as does the pathogenicity of specific infectious agents (25). Phylogenetic proximity, as well as the ability of the agent to mutate or to cross-infect non-host species, has meant that viral, bacterial, mycotic and chlamydial agents which had not been known to cause disease in certain species are capable of pathogenicity. Some diseases affect wildlife first and later cross over into domestic animals or humans. Diseases which occur in domestic animals may spread rapidly, due to the intensive nature of most commercial production models. Confinement of wild animals for captive propagation or exhibition also propitiates a more rapid and potentially devastating spread of disease.

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The ability both to detect and to determine the pathogenicity of an infectious agent is critical to a disease monitoring programme (25). In domestic animals, the economic implications to agriculture mean that considerable resources and research are dedicated to detecting, classifying, treating and preventing disease. Unfortunately, the only means of detecting most infectious diseases are those which have been developed specifically for domestic animals. The use of the same tests for non-domestic species is not always valid, but may be the only means of evaluating the disease status of captive wild animals.

Numerous factors affect disease transmission in captive populations. Animals in zoos are often in environments which differ from their native habitats. Zoo animals may be housed in close proximity to species with which they would never have contact in the wild. Management practices can significantly influence disease processes.

Species Survival Plans (SSPs) in North America — and comparable programmes in other parts of the world — address the management issues related to maintaining populations of endangered species in captivity. Health issues are an important aspect of these programmes, particularly in species intended for return to the wild. (In the opinion of the authors, the ‘wild’ no longer exists. As the human population burgeons towards 6 billion, any wildlife which is not already managed may need to be managed to survive.)

**SPECIES SURVIVAL PLAN PROGRAMMES**

The SSP is a co-operative population management and conservation programme administered by the American Zoo and Aquarium Association (AZA) (32). Originally conceived in an effort to establish programmes of co-operation between facilities in North America, SSPs now assume broader conservation goals (Appendix I). SSPs facilitate the maintenance of a genetically-viable and demographically-stable population of a given species in captivity. They also support species preservation by raising funds for habitat and field conservation efforts. In addition, SSPs educate the public and foster basic veterinary, nutritional and reproductive research (32).

It has been suggested that conservation programmes should maintain populations of sufficient size and genetic diversity to ensure that there is a 95% chance of species surviving for 100 years (3). The maintenance of metapopulations (subpopulations in geographically-distinct areas) is a logical strategy to reduce the possibility of a single epidemic annihilating an entire population (3). In essence, SSPs support both of these fundamental conservation strategies.

A total of 74 SSPs have been established for 121 species (59 mammals, 17 birds, 7 reptiles and amphibians, 34 fish and 4 invertebrates) (Appendix II). Each SSP has a species coordinator and a management group, who are responsible for developing a ‘Master Plan’. The Master Plan outlines long-range goals, management strategies and breeding recommendations for animals in participating institutions. The ‘studbook’ keeper determines the genealogy of each animal in the SSP population and continually updates a database of births, deaths and animal locations. The Small Population
Management Advisory Group provides technical assistance, with genetic and demographic analyses, to assist the SSP in determining appropriate pairings. In addition to the Master Plan, each SSP formulates a Five-Year Action Plan which identifies needs for research, education, field conservation and related projects.

While SSPs focus on a single species, other AZA programmes have broader scope. Taxon Advisory Groups (TAGs) concentrate on the relationships and comparisons between related species. They are responsible for developing a regional collection plan for a given taxon. In addition, TAGs establish priorities for management, research and conservation action. Recommendations for priority species for new studbooks and SSPs are often initiated by these groups. There are currently 41 TAGs in operation. Fauna Interest Groups (FIGs) were established to coordinate AZA programmes in specific geographical areas. There are currently seven FIGs (covering Brazil, Mesoamerica, South-East Asia, the West Indies, Zaire, Madagascar and the Cuban Amazon).

Scientific Advisory Groups (SAGs) are comprised of zoo professionals and outside experts who research specific topics. The SAGs serve as technical advisors to SSPs and other AZA programmes. Established SAGs cover a variety of areas, including behaviour and husbandry, reintroduction, veterinary science, contraception, nutrition, small population management and genome banking.

To date, 56 SSPs and 26 TAGs have veterinary advisors. The Veterinary Scientific Advisory Group has established the following guidelines for SSP veterinary advisors (P.L. Wolff, personal communication):

1. Identify the major medical problems of the species/taxa.

2. Participate in the development and distribution of medical protocols through the species/taxa husbandry manual, studbook, etc.

3. Identify specialists in the areas affecting the health and well-being of the species. Assist in coordinating data collection efforts between other AZA Science Advisors, scientific specialists, wildlife collection managers and veterinarians.

4. Provide regular (at least annual) reports to the Species Management Group and the chair of the Veterinary Science Advisory Committee.

5. Advise the SSP/TAG on animal welfare, guidelines for surplus animals, and significant Federal legislation as it pertains to captive breeding, movement and/or reintroduction.

6. Facilitate the development of centralized serum and tissue banks.

7. Act as a reviewer for proposed research protocols and methods pertaining to the species/taxa. The SSP/TAG Management Group must be advised on the value of such proposals, and the potential health and welfare issues associated with them.

8. Act as a reviewer for the medical portion of any surveys sent out through the SSP/TAG or received by the SSP/TAG.

9. Co-operate closely with the other Scientific Advisors for the species.
Numerous factors influence the actual or potential transmission of disease in captive populations of animals. Institutional practices and policies for animal husbandry and management may favour or inhibit disease occurrence. A sound knowledge of disease epidemiology, prevention and treatment is of paramount importance. Personnel at every level of animal contact must understand basic concepts of sanitation and disease transmission. Detailed record-keeping is essential for the control of disease within individual facilities and for the sharing of information between institutions. Local, national and international animal health regulatory agencies also affect collections of captive wild animals. Animal movement is subject to regulations at various levels.

Impact of institution management practices on disease transmission

As signs of disease in wild animals are often masked, even at advanced stages, preventive medicine has become the hallmark of zoo veterinary practice. Preventive medicine includes procedures to screen for existing disease (tuberculin testing and faecal examination) or to provide pre-exposure protection against disease (vaccination, heartworm prophylaxis). Quarantine is one of the most important aspects of preventive health care programmes (16). Other aspects of preventive health care include nutritional evaluation, and routine health monitoring through physical examination. Basic sanitary practices must be in place to prevent transmission of infectious agents — either directly or via fomites — between different parts of the facility. Personnel must receive hygiene training, and must understand the importance of enclosure cleanliness, hand-washing and the use of footbaths. A health monitoring programme for staff can prevent disease transmission to or from collection animals (9, 28). Thorough ante-mortem and post-mortem investigation, and documentation of disease occurrences are essential.

A number of documents have been developed by the North American zoo community to address disease issues and provide institutional standards. Guidelines for zoo and aquarium veterinary medical programs and veterinary hospitals have been adopted by the Veterinary Standards Committee of the American Association of Zoo Veterinarians (AAZV) (17). These guidelines outline recommendations for veterinary cover, hospital facilities, support personnel, preventive medicine, animal shipments, and diet and husbandry reviews. Detailed recommendations concerning basic medical care, quarantine, vaccination, parasite control and husbandry procedures have been formulated by the Infectious Disease Committee of the AAZV (18). The AZA has developed recommended quarantine procedures for member institutions (21). Zoonotic diseases are addressed in the AAZV Guidelines, as well as in Zoonotic diseases, a pamphlet published by the American Association of Zoo Keepers (7).

Risk assessment

The evaluation of benefits and risks (risk assessment) to aid decision-making on management actions has recently become a popular conservation planning tool (4). Ecological variables (including disease) are identified and applied to mathematical
models, which then predict the probability of both extinction and loss of genetic diversity. Effects of individual risk factors can be quantified, as well as the interaction between variables.

A system has also been proposed to establish a hierarchy of disease risks in captive populations intended for release (25). Based on the Mace/Lande system for categorizing species by degree of extinction, parameters such as ‘pathogenicity’ and ‘prevention availability’ are used to determine the degree of threat. These criteria can then be used to categorize diseases as ‘high risk’, ‘low risk’ or ‘no risk’. Recommendations are given for the investigation of high priority diseases (25).

Role of government agencies concerned with health issues

Legislation specific to wildlife is generally concerned with conservation of species. Such legislation affects the possession, exhibition and movement of certain animals (30). Animal health laws, for the most part, focus on diseases which present a threat to commercial agricultural industries or to humans (11). In many cases, animal health laws neither include nor exclude wild animals. Decisions concerning wild animals may be made, on a case-by-case basis, by the authorizing agent in charge. Coordination with government agencies is critical.

Domestic animal health programmes and laws may present a confusing situation when wildlife species become involved. Screening tests and control methods routinely used for domestic animals may not be valid in other species. As an example, tuberculosis continues to provide a diagnostic challenge in non-domestic hoofed animals, and has affected both captive propagation and reintroduction programmes (8, 12, 14). Improved testing procedures are also needed for bluetongue, epizootic haemorrhagic diarrhoea and equine babesiosis (15).

Record-keeping procedures used to monitor diseases

Within an institutional framework, medical records are used to document disease occurrence and treatments administered to individual animals. Many zoos evaluate morbidity and mortality annually as a tool for management and health care practices. For the most part, information is shared in professional meetings, through journal publications, or informally between colleagues discussing difficult cases. In the continued movement of captive-held animals to other facilities or to the wild, access to current information on disease issues is critical. Veterinary advisors to SSPs need comprehensive medical information to help them in making decisions on health issues (29). Such data exist for only a few species.

The benefits of a standardized, computerized medical record-keeping system cannot be overstated. In addition to allowing analysis of data from an institution, a standardized system also enables information sharing between facilities. Prospective medical information can be accessed, analyzed and made available to veterinary advisors in a timely manner.
The most widely-used system in this field is the Medical Animal Record Keeping System (MedARKS). At present, there are 310 MedARKS users in 38 countries. Designed by zoo veterinarians, the MedARKS system has modules for clinical notes, treatment, anaesthesia, parasitology, clinical pathology, and serum and tissue banking. Additional modules are in preparation.

Disease monitoring is a component of several SSPs and will be a part of all SSPs if the recommendations of the Veterinary Scientific Advisory Group are adopted. The Cheetah SSP is exemplary in utilizing an interdisciplinary team (a nutritionist, a geneticist, a clinical veterinarian, a pathologist, a reproductive physiologist, and a behaviourist) (20). Disease/pathology surveys have been conducted for black rhinoceroses \textit{(Diceros bicornis)} (E. Miller, personal communication), cheetahs \textit{(Acinonyx jubatus)} (13, 24), maned wolves \textit{(Chrysocyon brachyurus)} (23), red pandas \textit{(Ailurus fulgens)} (22) and prosimians (6). Detailed retrospective studies have been performed for orang-utans \textit{(Pongo pygmaeus)} (31) and elephants (19).

The need for continuous surveillance of captive animals, especially those intended for reintroduction, is clear. The components of a disease monitoring programme have been identified, and may be listed as follows (25):

\begin{enumerate}
\item \textbf{a)} Collection of consistent biological materials and data:
  \begin{enumerate}
  \item necropsy protocols for correct tissue sampling for pathology, virology and bacteriology
  \item protocols for sampling of faeces, blood and pelage for parasites
  \item physical examination protocols.
  \end{enumerate}
\item \textbf{b)} Participation of all zoological parks.
\item \textbf{c)} Consistent evaluation of biological materials:
  \begin{enumerate}
  \item identification of experienced comparative pathologists
  \item identification of appropriate ancillary laboratories
  \item protocols for bacterial and viral culture
  \item protocols for parasitological examination.
  \end{enumerate}
\item \textbf{d)} Centralization of results:
  \begin{enumerate}
  \item development of computer-based programs for data input, analysis and retrieval
  \item integration of programs with existing zoo animal inventory and medical programs
  \item periodic review of databases by veterinary advisors.
  \end{enumerate}
\item \textbf{e)} Communication of results to zoo and wildlife communities.
\end{enumerate}
Several initiatives have emerged to address the monitoring, investigation and surveillance of disease in captive and free-ranging wildlife. An international system has been proposed, modelled on a system developed by the American Committee on Arthropod-borne Viruses. Epidemiology, laboratory diagnosis and an information bank/database are key components of the system (26).

It has also been proposed that the Office International des Epizooties (OIE) collect information on wildlife diseases. The OIE coordinates the control of contagious domestic animal diseases through a global network of 143 Member Countries. Review of international wildlife movement regulations, and standardization of diagnostic procedures and vaccination protocols are additional aspects of this initiative (34).

Similar recommendations were made by a working group formed during the International Conference on Implications of Infectious Disease for Captive Propagation and Reintroduction of Threatened Species (1; see below).

**PROTOCOLS FOR REGIONAL SPECIES CONSERVATION PROGRAMMES**

At present, there are virtually as many recommendations on captive species management as there are SSP programmes. Indications by advisors range all the way from the extremely simple and intuitively correct need for pre-release quarantine, observation and physical examination, to protocols for specific tests and procedures involving individual members of certain species. FIGs have historically covered too broad a range of species to make specific recommendations. The goal of FIGs is to promote the environmental well-being of entire regions.

Some TAG protocols, and all SSP protocols, recommend procedures which are common in institutions dealing with animals. General practices include regular weighing, quarantine, and monitoring for pathogens. Advisors to TAGs identify disease risks and recommend testing procedures. In some cases, recommended protocols have been so broad that they cover entire orders or classes of animals involved in captive breeding situations (16, 35).

Many conservation programmes have similar components. This was recognized at an early stage in the implementation of single species conservation and reintroduction efforts. The need was recognized for universal methods of assessing, evaluating, monitoring and preventing the transmission of disease to naive wildlife by reintroduced species. At the International Conference on Implications of Infectious Disease for Captive Propagation and Reintroduction of Threatened Species – held in Oakland, California (USA) from 11 to 13 November 1992 – five working groups of recognized experts in the field of wildlife disease met and developed guidelines, recommendations and manuals designed to standardize data collection through established protocols to be used by Regional Species Conservation Programmes (RSCPs). The working group dedicated to the monitoring, investigation and surveillance of disease in captive wildlife produced a manual (10), consisting of four major components. The first part of this manual includes the protocols for the monitoring and surveillance of disease in species involved in captive breeding and
reintroduction programmes. The three remaining sections present guidelines for developing a central disease database, criteria for risk assessment in determining the threat of infectious diseases in reintroduction programmes, and the interpretation and reporting of diagnostic tests, respectively. Details are given below of the specific protocols recommended in the first section.

The RSCP protocols are universal in application. They are presented as generic guidelines to be tailored to each species or taxon. These detailed protocols enable optimal data collection.

The RSCPs emphasize the importance of designating a veterinary advisor for each conservation plan. The functions of the advisor are clearly indicated in the list of components for a disease monitoring plan given above (see ‘Record-keeping procedures used to monitor diseases’ above) (25).

In many cases, information on clinical and subclinical disease is best obtained through tissue examination of dead individuals of the group of interest. The RSCP Necropsy Protocol Worksheet can be altered to fit the needs of each programme. This worksheet covers all major classes of animals involved in conservation projects, including neonates and fetuses. The RSCP Necropsy Protocol is presented in Appendix III. A full tissue collection list is presented in Appendix IV.

Animals considered for reintroduction must be carefully scrutinized during captivity, and must undergo a stringent pre-release quarantine. The protocol detailed in Appendix V is designed to maximize the collection of data on each animal considered for release. The protocol should merely serve as a starting point for the RSCP veterinary advisors. Specific tests should reflect the extent of knowledge on pertinent disease processes in captivity and in the wild. Pre-release screening does not preclude the possibility of disease introduction to naive wild populations; it only minimizes such occurrences within the limitations of current knowledge.

Veterinary advisors to RSCPs are responsible for implementing comprehensive animal health programmes. Veterinary involvement in reintroduction planning should be an essential part of a complete programme (5). It is suggested that the following components be included (10):

- quarantine
- vaccination
- disease surveillance (parasitology, serology, haematology, biochemistry, tuberculin testing, tissue culture, banking of biological materials)
- physical examination
- personnel health screening
- pest control
- review of husbandry methods and facilities
- medical record-keeping
- regulatory compliance.

A veterinary advisor can follow an established protocol in reporting all collected information to the RSCP (Appendix VI). Once again, the form is generic and can be tailored to each species or taxon.
Standards of practice must be developed which ultimately improve the animal health situation. Species- or taxon-specific guidelines may safeguard against inadvertent introduction of disease into wild populations. These initiatives clearly define what needs to be done. Further coordination and co-operation are required in performing these tasks.

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Résumé : Les « Plans de survie » (Species Survival Plans) d’espèces animales vivantes en Amérique du Nord et d’autres programmes similaires ailleurs dans le monde sont confrontés aux problèmes de gestion liés au maintien en captivité d’animaux appartenant à des espèces menacées d’extinction. Il apparaît nécessaire d’harmoniser les méthodes d’évaluation, de surveillance et de prévention afin d’éviter que les espèces réintroduites ne transmettent des maladies à la faune sauvage, qui n’est pas protégée. Les auteurs présentent des protocoles normalisés pour évaluer le statut sanitaire des animaux en captivité destinés à être relâchés dans la nature.


* *


Resumen: Los «Planes de Supervivencia de Especies» (Species Survival Plans) en América del Norte, al igual que los programas semejantes en otras partes del mundo, tratan los aspectos de manejo relativos al mantenimiento en cautividad de poblaciones de especies amenazadas. Se ha constatado la necesidad de métodos universales de estimación, evaluación, monitoreo y prevención de la transmisión de enfermedades a la fauna salvaje a partir de especies reintroducidas. Los autores presentan protocolos estandarizados para la evaluación del estado sanitario de los animales en cautividad que van a ser liberados al medio salvaje.


* *

* *
Appendix I

Species Survival Plan: Mission statement

(2)

The mission of the American Zoo and Aquarium Association’s Species Survival Plan Program is to help ensure the survival of selected wildlife species.

The mission will be implemented using a combination of the following categories:

- Organize scientifically managed captive breeding programs for selected wildlife as a hedge against extinction.
- Co-operate with other institutions and agencies to ensure integrated conservation strategies.
- Increase public awareness of wildlife conservation issues.
- Conduct basic and applied research to contribute to our knowledge of various species.
- Train wildlife and zoo professionals.
- Develop and test various technologies relevant to field conservation.
- Reintroduce captive-bred wildlife into restored or secure habitat as appropriate and necessary.

*     *

Appendix II

Species Survival Plans

Mammals

Addax (Addax nasomaculatus)      Cheetah (Acinonyx jubatus)
African wild dog (Lycaon pictus)  Chimpanzee (Pan troglodytes)
Arabian oryx (Oryx gazella leucoryx)  Clouded leopard (Neofelis nebulosa)
Asian small-clawed otter (Aonyx cinerea)  Cotton-top tamarin (Sanguinus oedipus)
Babirusa (Babirousa babirussa)  Drill (Papio [Mandrillus] leucophaeus)
Barasingha (Cervus [Rucervus] duvauceli)  Elephant (Loxodonta spp., Elephas maximus)
Black and white colobus (Colobus polykomos/angolensis/guereza/abyssinicu)  Gaur (Bos [Bibos] gaurus)
Black-footed ferret (Mustela nigripes)  Giant panda (Ailuropoda melanoleuca)
Black lemur (Lemur macaco)  Gibbon (Hylobates spp.)
Black rhinoceros (Diceros bicornis)  Goeldi’s monkey (Callimico goeldii)
Bonobo (Pan paniscus)  Golden lion tamarin (Leontopithecus rosalia/roeselai)
Chacoan peccary (Tayassu catagonus wagneri)  Greater one-horned Asian rhinoceros (Rhinoceros unicornis/sondalicus)
Mammals (contd)

Grévy’s zebra (*Equus [Dolicholippus] grevyi*)
Hartmann’s mountain zebra (*Equus [Hippotigris] zebra hartmannae*)
Jaguar (*Panthera onca*)
Lions (*Panthera leo*)
Lion-tailed macaques (*Macaca silenus*)
Lowland gorilla (*Gorilla gorilla*)
Maned wolf (*Chrysocyon brachyurus*)
Mangabey (*Cercocebus spp.*)
Mexican grey wolf (*Canis lupus baileyi*)
Mongoose lemur (*Lemur mongoz*)
Okapi (*Okapia johnstoni*)
Orang-utan (*Pongo pygmaeus*)
Pygmy hippopotamus (*Choeropsis liberiensis*)
Pygmy loris (*Nycticebus pygmaeus*)

Birds

Bali mynah (*Leucospar rothschildi*)
Cinereous vulture (*Aegypius monachus*)
Condor (*Gymnogyps californianus/ Vultur gryphus*)
Congo peafowl (*Afroparo congensis*)
Crane (*Gruidae*)
Greater hornbill (*Buceros bicornis*)
Guam rail (*Gallirallus [philippines] owstoni*)

Reptiles and Amphibians

Aruba island rattlesnake (*Crotalus sp.*)
Chinese alligator (*Alligator sinensis*)
Cuban crocodile (*Crocodylus rhombifer*)
Dumeril’s ground boa (*Acrantophis dumerili*)

Fish

Haplochromine cichlids (34 species) (*Haplochromis* spp.)

Invertebrates

Partula snail (*Partula spp.*)
# Appendix III

Regional Species Conservation Programme
Necropsy Protocol Worksheet

<table>
<thead>
<tr>
<th>Common name:</th>
<th>Genus/Species:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isis #:</td>
<td>Studbook #:</td>
</tr>
<tr>
<td>Date of birth:</td>
<td>Age:</td>
</tr>
<tr>
<td>Date of death:</td>
<td></td>
</tr>
<tr>
<td>Gross examination by:</td>
<td>Histopathology by:</td>
</tr>
<tr>
<td>Institution/Owner/Address:</td>
<td></td>
</tr>
</tbody>
</table>

History (include clinical signs, treatments, ante-mortem test results, diet, circumstances of death and quarantine status; attach copy of medical record): __________

## GROSS EXAMINATION WORKSHEET

### General condition
(nutritional condition, physical condition, body score, skin):

- **Musculoskeletal system** (bone, joints, muscles):
- **Body cavities** (fat stores, abnormal fluids):
- **Haemolymphatic system** (spleen, lymph nodes, thymus, bursa of Fabricius):
- **Respiratory system** (nasal cavity, larynx, trachea, lungs, regional lymph nodes, air sacs):

- **Cardiovascular system** (heart, pericardium, great vessels):
- **Digestive system** (mouth, teeth, oesophagus, stomach, intestines, liver, pancreas, mesenteric lymph nodes):
- **Urinary system** (kidneys, ureters, urinary bladder, urethra):
- **Reproductive system** (testis/ovary, uterus, oviduct, vagina, cloaca, penis, prepuce, accessory glands, mammary glands, placenta):
- **Endocrine system** (adrenals, thyroid, parathyroids, pituitary):
- **Nervous and sensory systems** (brain, spinal cord, peripheral nerves, eyes, ears):

## GROSS DIAGNOSIS
List each lesion separately; include organ, lesion type, distribution, severity, etc.:

## LABORATORY DIAGNOSIS
List samples submitted (bacteriology, viral, parasitology, haematology, etc.) and attach results:

* *
Appendix IV

Regional Species Conservation Program Necropsy Protocol
Tissue Collection List

Instructions

Take **duplicate sets of tissues for the regional species conservation program pathologist**. Preserve the listed tissues in 10% buffered formalin at a ratio of one part tissue to ten parts formalin. Tissues should be no thicker than 1 cm. **Include sections of all lesions** and samples from all tissues listed. For **embryos or neonates**, also include the information in the **neonatal protocol**.

Tissues to sample

**Mammals**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>Large intestine</td>
</tr>
<tr>
<td>Lungs</td>
<td>Skeletal muscle</td>
</tr>
<tr>
<td>Trachea/oesophagus</td>
<td>Bone/bone marrow</td>
</tr>
<tr>
<td>Thymus</td>
<td>Tongue</td>
</tr>
<tr>
<td>Thyroid/parathyroids</td>
<td>Adrenal</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Kidneys</td>
</tr>
<tr>
<td>Spleen</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>Eyes</td>
<td>Uterus/ovary</td>
</tr>
<tr>
<td>Liver/gall bladder</td>
<td>Testis/epididymis/prostate</td>
</tr>
<tr>
<td>Stomach</td>
<td>Brain/nervous tissue</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Skin</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
</tr>
</tbody>
</table>

**Birds**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>Liver</td>
</tr>
<tr>
<td>Lungs</td>
<td>Crop</td>
</tr>
<tr>
<td>Trachea</td>
<td>Povventriculus</td>
</tr>
<tr>
<td>Air sacs</td>
<td>Ventriculus</td>
</tr>
<tr>
<td>Thymus</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Spleen</td>
<td>Large intestine</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Skeletal muscle</td>
</tr>
<tr>
<td>Eyes</td>
<td>Bone/bone marrow</td>
</tr>
</tbody>
</table>
**Birds (contd)**

<table>
<thead>
<tr>
<th>Organs</th>
<th>Organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal</td>
<td>Testis/ovary</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Oviduct</td>
</tr>
<tr>
<td>Bursa of Fabricius</td>
<td>Thyroid/parathyroid</td>
</tr>
<tr>
<td>Skin with feathers</td>
<td>Brain/nervous tissue</td>
</tr>
</tbody>
</table>

**Reptiles and amphibians**

<table>
<thead>
<tr>
<th>Organs</th>
<th>Organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>Large intestine</td>
</tr>
<tr>
<td>Lungs</td>
<td>Skeletal muscle</td>
</tr>
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<td>Trachea</td>
<td>Bone/bone marrow</td>
</tr>
<tr>
<td>Thymus</td>
<td>Adrenal</td>
</tr>
<tr>
<td>Skin</td>
<td>Kidneys</td>
</tr>
<tr>
<td>Spleen</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>Eyes</td>
<td>Testis/ovary</td>
</tr>
<tr>
<td>Liver</td>
<td>Oviduct</td>
</tr>
<tr>
<td>Stomach</td>
<td>Brain/nervous tissue</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Thyroid/parathyroid</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
</tr>
</tbody>
</table>

**Essential frozen tissue**

If possible 10 g of liver, brain and kidney should be stored, in addition to ante-mortem serum and plasma, in an appropriate container at \(-70^\circ\)C.

**Additional protocol for neonatal or fetal necropsy**

1. Fix the umbilical stump and surrounding tissues.
2. Examine for malformations (cleft palate, deformed limbs).
3. Assess hydration (tissue moistness) and evidence of nursing/eating (food or milk in stomach).
4. Determine whether breathing occurred. (Do the lungs float in formalin?)
5. Examine placenta.

(For avian and reptilian embryos, open the coelomic cavity and fix the entire embryo. Include egg shell and membranes if available.)

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Appendix V

Protocol for minimum pre-introduction quarantine

(10)

Date: ______________________________

Animal identification: accession #: ________ Transponder #: ________
Band #: ________ Genetic identification: ________ Other identification: ________

Current drug exposure (including contraceptives): __________________________

Facility standards

Length of quarantine: ______________________________
Sanitation and hygiene requirements: ______________________________

Required testing/biomaterials collection for species

Serology (date, test, results): ______________________________
Faecal (method, results): ______________________________
Culture (sites, results): ______________________________
Biomaterials: ______________________________

Baseline physiological data

Temperature (°C): ________ Weight (kg): ________
Pulse/heart rate (beats per minute): ________ Respiration (breaths per minute): ________

Physical examination

Ears/eyes/nose/throat: ______________________________
Heart/lungs: ______________________________
Abdomen: ______________________________
Musculoskeletal: ______________________________
Urogenital: ______________________________
Dermatological: ______________________________
Other: ______________________________

Clinical laboratory tests performed

Complete blood count: ________ Biochemistry: ________ Urinalysis: ________ Faecal: ________
Vaccine: modified live vaccine/killed (name brand): ______________________________
Vaccination date: ________ Serial #: ______________________________

Additional required tests (might include):

Radiographs: ________ Tuberculin testing: ________ Immune status: ________ Other: ________

Pre-release standards – the following tests must be negative or within acceptable limits for this species to be released (for example):

Negative tuberculin test: ________ White blood cell count no greater than: ________
3 negative faecal checks: ________ ________ ________ ________ ________ ________
Negative faecal culture: ________ Negative blood parasites: ______________________________

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Appendix VI

Regional Veterinary Advisor's Reporting Protocol

I. Necropsy summary:
   a) Total number of deaths (sex and age demographics)
   b) Cause of death
   c) Significant lesions and prevalence based on year and total
   d) Test results.

II. Significant morbidity:
   a) Total numbers
   b) Age and sex predilections
   c) Presenting signs
   d) Therapeutics.

III. Significant test results: including means and ranges (if significant number of normal individuals).

IV. Other significant activities:
   a) Genetics
   b) Nutrition
   c) Reproduction
   d) Other research.

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REFERENCES


