

**QUARANTINE AND HEALTH SCREENING PROTOCOLS FOR WILDLIFE
PRIOR TO TRANSLOCATION AND RELEASE INTO THE WILD**

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Quarantine and Health Screening Protocols for Wildlife prior to Translocation and Release into the Wild

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FOREWORD

The release of animals, whether it be for translocation from one wild population to another, the introduction of captive-borne animals into a natural wild population, or the return of rehabilitated animals into the wild after varying periods of time in captivity, have become commonplace in recent years. Such releases are not without consequences and must be considered in terms of the level of risk of disease transfer. Once ignored and little understood, it is now widely recognised that these releases must not be regarded as consisting of just a single animal, but rather of a package of organisms, including those viruses, bacteria, protozoa, helminths and arthropods, which any single animal may harbour. Any of these organisms may become pathogenic under stressful situations, affecting not only the released animal but equally important, other animals, including humans, in the release environment.

In an effort to address the disease considerations of wild animal releases, Michael Woodford has assembled information regarding quarantine, screening procedures and treatment/vaccination suggestions for mammals, birds, reptiles, amphibians and fish. These protocols are comprehensive and represent the ideal, given our current level of understanding of the potential risks of the various organisms that may be carried by any single animal. We are reminded that once an animal has been released into the wild, it is rarely, if ever, possible to recover that animal or the potential pathogens it may be carrying. Thus it is incumbent upon all who are charged with the release of wildlife to follow stringent guidelines that will help to ensure the merit and wisdom of releasing such wildlife.

“Quarantine and Health Screening Protocols for Wildlife Prior to Translocation to Release into the Wild” is an excellent reference source and will enable the veterinarian to make rational decisions regarding release based on currently available science. Michael Woodford and his fellow contributors are to be congratulated for their thoughtful approach to this subject.

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QUARANTINE AND HEALTH SCREENING PROTOCOLS FOR WILDLIFE PRIOR TO TRANSLOCATION AND RELEASE INTO THE WILD

1. INTRODUCTION

In recent years the translocation and release into the wild of wild-caught and captive-bred wild animals (mammals, birds, reptiles, amphibians and fish) has become a common practice, ostensibly for rehabilitation or conservation purposes.¹ These wild animals comprise many varied taxa and the objectives of translocation and release may include:

- (1) reintroducing a species that has become extinct in its natural range;
- (2) restocking or reinforcing a population which has become depleted; and
- (3) rehabilitating wild animals and birds which have been illegally captured and subsequently confiscated by Customs or national wildlife authorities. Welfare organisations also receive sick and injured wild animals from the public and some of these can be restored to health and released.

Each year very large numbers of wild animals also undergo both local and transcontinental translocation for release in new and strange habitats for sporting purposes. For example, hares (*Lepus europaeus*) are regularly translocated from Argentina to France and from Eastern Europe to Italy.²

It is now widely recognised by wildlife veterinarians that every wild creature that is the subject of a translocation or rehabilitation release must not be regarded as just a single animal but rather as a “package” containing an assortment of potentially dangerous viruses, bacteria, protozoa, helminths and arthropods, any of which may become pathogenic in a new situation, involving stressed individuals in a changed environment.³

Translocation of an animal and its potential pathogens, over even a short distance, may threaten the health of indigenous wild species, domestic livestock or humans. In addition, the effects of stress on the immune system of animals while held in captivity pending translocation and release may increase this risk, unless well managed. However, the risk can be assessed in advance and substantially reduced if timely veterinary precautions are taken.

These precautions will include: a clinical evaluation of the health status of the source animals and those at the translocation destination, a period of quarantine, appropriate health screening procedures, a consideration of the legal and veterinary restrictions on translocation of wild animals to and from certain geographic areas or populations, and when necessary, pre-release treatment and immunisation. It should be remembered that not only should the translocated animals undergo health screening but so also should the indigenous wildlife in the reception area.^{4, 5}

Translocation and Reintroduction Policy

The reader is referred at an early stage to two important policy statements and guidelines. The first was prepared by the IUCN Species Survival Commission in collaboration with the Commission on Ecology and the Commission on Environmental Policy, Law and Administration.² This document, published in 1987, sets out IUCN's position on the Translocation of Living Organisms, covering introductions, re-introductions and re-stocking.

The second document, entitled IUCN Guidelines for Re-introductions, has been prepared by the IUCN/SSC Re-introduction Specialist Group in 1998,¹ and consists of specific policy guidelines designed to ensure that translocations and re-introductions achieve their intended conservation benefit and are not responsible for any adverse consequences.

Both these important Policy Statements should be carefully studied before any living organism is translocated for whatever purpose.

Note: It must always be remembered that once a wild animal has been released into the wild it is very rarely possible to recover it or the potential pathogens it may have carried.

Evaluation of the health status of the animals and their source population

It is recommended that the attending veterinarian should obtain and review all available information concerning the health of the animals to be translocated, and where possible, that of their source population. Sources of such information could include published literature, unpublished necropsy and diagnostic records of local national veterinary laboratories, national departments of agriculture and colleges of veterinary medicine.

Disease surveillance protocols and information on the occurrence of domestic animal (and some wildlife) diseases can be obtained from the annual **FAO Animal Health Yearbook**, the **OIE 'Animal Health in 1997'** publication and for Europe, from **Faune Sauvage d'Europe** (*Informations Techniques des Services Veterinaires*), and from **Bulletin d'Information sur la Pathologie des Animaux Sauvages en France (BIPAS)**. Information on wildlife diseases can be obtained from a variety of sources including national or regional wildlife authorities, national veterinary services and international veterinary information organisations such as the **IUCN/SSC Veterinary Specialist Group** or the **OIE Working Group on Wildlife Diseases** and for zoonoses, the **Institut Pasteur** in Paris (and in other countries), and the **World Health Organisation** in Geneva, may be consulted. (See appendix for organisation addresses).

Consultation with veterinarians in the source area should be sought to determine if any diseases of concern are known to be enzootic in domestic livestock or wildlife in the source area. In some cases it may be desirable and possible to sample the wild animal populations in the source and destination areas by serology and autopsy. Positive serologic results may warrant further investigations to detect the presence of active infections. Opportunistic sampling of hunter-harvested animals, road kills and rehabilitation centre casualties may also be undertaken.

The quarantine protocol, the list of screening procedures and the treatment/vaccination suggestions which follow are as comprehensive for each taxon as possible and represent those procedures that should ideally be performed when adequate funding, laboratory services and veterinary expertise are available. It is well recognised that this may not always be the case and in these circumstances close liaison between the conservation authorities and their veterinary advisors must be maintained so that the health hazards, which are implicit in all wild animal translocations, can be objectively assessed and where possible, minimised. It may well be that the veterinary risks which are judged to accompany a given translocation proposal are so great that in no circumstances should that operation be permitted to proceed.

In some cases it may not be possible to adhere to these guidelines because the health and welfare of the species to be released will be jeopardised if the animal is held in close confinement for a long period. For example, some species of New Zealand's small birds (robins) will die if confined for as little as 24 hours. In these cases, the risks of translocation must be judged against the benefits of releasing the animals.

Quarantine ^{7, 8, 15}

The purpose of quarantine is to allow the detection of those animals, which may be incubating a disease with a short incubation period and also to detect the clinical signs of diseases with a longer incubation period.

When captive wild animals are to be returned to the wild, for whatever reason, they should be isolated from all other animals not of the same consignment, in suitable secure premises, approved by a veterinarian, for at least 30 days immediately before shipment for release.

For many species pre-shipment quarantine may be an international requirement, especially if the origin of the animals is another country. The recipient country may also have regulations regarding post-shipment quarantine.

Quarantine premises must be appropriate for the species concerned and must take into account the epidemiological situation in the region or country of origin and that in the destination area. Thus, very finely netted enclosures may need to be provided where the vectors of vector-borne diseases (e.g. African horse sickness and bluetongue) must be excluded. The premises must allow adequate visual access and permit clinical examination, sampling and when necessary, chemical rather than physical restraint. Isolation from all possible sources of infection must be absolute.

The main requirement is that a group of wild animals (or a single animal) under quarantine must be isolated from all others of its own or closely related species and if possible, from all other species, wild and domestic, too. In the case of primates this will mean isolation from all humans other than the attendants.

Quarantine for all species should be for a **minimum of 30 days** and should be under the supervision of a veterinarian. The 30 day minimum quarantine period may need to be extended in the case of diseases which exhibit a long and often unpredictable incubation period (e.g. rabies, tuberculosis) and in other special circumstances.¹⁶

For **cervids** in USA, guidelines for quarantine are linked to testing requirements for brucellosis and bovine tuberculosis. Fulfillment of these guidelines requires that animals with no prior testing history be held in their State of origin, in isolation, for at least **93 days**. This minimum period allows for the required **90 days** interval between two consecutive tuberculin tests and an additional three days for reading the second test.

Test requirements for brucellosis, which comprise a negative test within **30 days** prior to shipment can be satisfied within this time frame.

Quarantine should always be conducted on an "All in, All out" basis. Should an animal undergoing quarantine in a group with others of the same species contract an infectious disease or test positive for an infectious disease while in quarantine, it must be removed from the group and placed in isolation. The whole group must then undergo a further 30 day, or longer, period of quarantine with appropriate testing. The length of the quarantine period will depend again on the incubation period of the disease concerned.

The attendant of specific groups of wild animals under quarantine must care for them alone and should not visit or come into contact with animals of similar taxonomic groups which may be undergoing quarantine in an adjacent domestic animal quarantine station. The attendant should also refrain from contact with all domestic animals, other exotic animals or contaminated foodstuffs, for the period of the quarantine.

Equipment used to feed and water the quarantined wild animals and to clean their enclosures must be used for these animals alone. Such equipment must be regularly disinfected with an appropriate disinfectant, designated by the attending veterinarian.

All dung-soiled bedding and discarded foodstuffs must be disposed of in a hygienic manner, preferably by burning.

Precautions must be taken to minimise the risk of exposure of the quarantine staff to zoonotic diseases that may be present in the newly acquired animals. These precautions should include the use of disinfectant foot baths, wearing of appropriate protective clothing, masks and gloves and minimising physical exposure to some species, e.g. primates by the wearing of face masks and by the use of chemical rather than physical restraint. Appropriate vaccination of the quarantine staff should be carried out.

During the quarantine period all animals, especially longhaired or woolly species, should be carefully checked to ensure that they are not inadvertent carriers of the seeds of plants exotic to the release area. Similarly they should be checked for infestation with ticks, which may be the vectors of diseases exotic to the release area.

Clinical examination and diagnostic testing

Unfortunately, most existing diagnostic serological tests have been developed for use in domestic livestock species and their validity for wildlife has not been ascertained. Furthermore, some assays require species-specific reagents, which probably are not yet commercially available. Many such assays have not yet been standardised. Consequently

great care must be taken when using these tests to determine whether an animal or population is infected and in making decisions regarding proposed translocation.⁹

A licensed veterinarian who has experience of the species concerned and of the likely diseases to which they may be susceptible, should carry out a thorough inspection of the animals destined for translocation.

At the first opportunity, each animal must be tagged, tattooed, or transpondered and in the case of primates, photographed, for permanent identification.

The inspector will note all visible conditions, including abnormal gait and behaviour, anorexia, diarrhoea, emaciation, salivation, polydipsia, hair loss and traumatic injuries.

It will be seen that some of the tests (checks) that should be carried out on animals while they are in quarantine prior to release are relatively simple and require little in the way of equipment. However, others will require access to a sophisticated laboratory, staffed by personnel experienced in the diagnosis of wildlife diseases. Interpretation of the results of the tests should be made by an experienced veterinarian who is familiar with the taxon concerned and the epidemiological situation in the areas of origin and destination.

On the basis of the results of the clinical examination and the laboratory tests, the veterinarian, in consultation and agreement with the relevant conservation authorities, shall decide whether the animal(s) shall be:

1. Translocated and Released
2. Definitely not Translocated or Released. (Maintenance in captivity or euthanasia may have to be considered)
3. Considered for Translocation and Release only after further investigation, quarantine and/or treatment.

Decisions on whether to add or omit a test, a treatment or a vaccination procedure must be left to the veterinarian overseeing the quarantine and the Translocation/Release.

In cases where a zoonosis may be translocated by a 'carrier' animal, it may be advisable to consult the **Centre for Disease Control (CDC)** in Atlanta, USA and/or the **World Health Organisation (WHO)** in Geneva, Switzerland and/or the **Institut Pasteur** in Paris, France.

Since animals are sometimes captive-bred on one continent for eventual release into the wild on another, international export and import regulations governing disease control may apply. These may dictate a pre- as well as a post-export protocol. Such animals are termed 'imported' in this booklet.

Finally, we must reiterate that it must always be remembered that once a wild animal has been released into the wild, it is very rarely possible to recover it or the potential pathogens it may have carried.

Restrictions on origin and destination

Examples of such restrictions are:

Artiodactyla should not be translocated to or from geographic areas or populations which are known to harbour wild or domestic ungulates infected with chronic wasting disease (Cervidae in USA), brucellosis, bovine tuberculosis, paratuberculosis, foot and mouth disease, rinderpest or septicaemic pasteurellosis.

White-tailed deer (*Odocoileus virginianus*) in USA carry a parasitic nematode (*Parelaphostrongylus tenuis*) in their meninges. This parasite, the intermediate host for which is a small land snail, is well tolerated by the deer. However, moose (*Alces alces*), are very susceptible, as are caribou (*Rangifer tarandus*) and most other cervids. Thus white-tailed deer should not be translocated into areas occupied by moose, caribou or other large Cervidae for which the meningeal worm can cause fatal disease. Conversely, moose, caribou and some other susceptible cervids would be unlikely to thrive in areas already occupied by white-tailed deer that are carrying *P. tenuis*.

Carnivores originating in rabies or *Echinococcus sp.* enzootic areas should not be released in rabies or *Echinococcus sp.*-free areas. Birds from Newcastle disease infected areas should not be released in Newcastle disease-free areas.

Due to the high sensitivity of otters (*Lutra lutra*) to polychlorinated biphenyls (PCBs), these animals should not be released into areas where the food web and environment is contaminated with these compounds.

Regulations of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) may apply and if so, should be strictly complied with.

Pre-release treatment

During the quarantine period, certain prophylactic measures may be instituted. Individual faecal samples or representative samples from large numbers of individuals housed in a limited area (e.g. birds of the same species in an aviary or frogs in a terrarium) should be collected at least twice and examined for endoparasites. Treatment, if not contra-indicated, should be prescribed by the attending veterinarian.

Animals should be free from therapeutic drugs for a minimum of one week prior to release into the wild. This precaution will prevent the drugs masking the signs of disease and will minimise the risk of the development of drug resistant organisms in the release environment.

Endo- and Ecto-parasites

Ideally, endoparasitic burdens should be evaluated on at least two occasions, three weeks apart, during quarantine. It may not be deemed necessary to administer anthelmintics if the revealed parasite load is light and if the species found are those likely to be encountered by

the released animals at their destination. However, heavy parasite burdens, which are often acquired in captivity, should be controlled before the animals are released and it may be desirable to eliminate a specific parasite if this is known not to occur in the proposed release environment. In addition, all animals should be evaluated for ectoparasites (especially sarcoptic mange, screw worm, warble fly and tick infestation) and treated accordingly.

When controlling endo- or ecto-parasites it is important to be clear what the objective is. In some circumstances, when the aim is to prevent the introduction of exotic, non-indigenous species, an attempt at complete elimination should be made. In another situation, the aim might be to reduce the parasite burden in order to minimise stress on the host. If the objective is complete elimination, (which may be difficult), then repeated follow-up checks may be required to ensure that this has been achieved and the animals should not be cleared for release until after the pre-patent period for the parasite in question has elapsed. Some parasitic worms (eg. *Echinococcus sp.*) will die out during the quarantine if the period is long enough (> two months) and reinfection is prevented.

Vaccination/Immunisation ¹⁵

The question of the desirability of pre-release vaccination should be carefully considered and the decision whether or not to immunise the animals to be released should be made by the attending veterinarian, after evaluating the immunological status of the animals held in quarantine and the likely challenge by enzootic and exotic disease agents upon release.

Vaccination of wild ungulates against enzootic diseases of contiguous domestic livestock, such as rinderpest or foot and mouth disease, may be indicated, as may vaccination against diseases known to be present in the release environment, such as clostridial diseases and anthrax.

It might be argued that immunisation of translocated animals against enzootic local diseases in the environment is contra-indicated because they would thus be afforded an unfair selective advantage over the resident wildlife. But this is not necessarily the case, because the resident wildlife would probably have been challenged under natural conditions when young, while under partial protection via colostral immunity, and would presumably have acquired a solid immunity later. In addition, usually only the founder generation of translocated animals would receive vaccine protection.

It is important to remember that some of the potential pathogens (e.g. *Bacillus anthracis* and *Clostridium botulinum*), which may occur in a release area are as much a part of the environmental biodiversity as are the animals to be released and have exerted selective pressures on unvaccinated wildlife for a very long time. These pressures have enabled the survivors to adapt to their environment and to resist the pathogenic challenge. One can thus probably afford to ignore these enzootic diseases, provided that the original foundation stock is vaccinated before release, so as to give it the greatest chance of survival while its numbers build up to the levels needed to preserve genetic fitness. Follow-up vaccination for subsequent generations would in any case be expensive, difficult to carry out and probably only justified for highly endangered species.

It is recognised that the advice given to repeat many of the vaccination procedures 'at suitable intervals' may not be possible when animals are held in quarantine for only 30 days. But animals bred in captivity for eventual release into the wild can usually be subjected to the recommended vaccination protocol. Animals born and raised in the wild may already be resistant to the enzootic pathogens and thus may not need vaccination. This is another reason to check relevant titres as part of the screening process and to evaluate each individual case.

When vaccination is considered, it is important to remember that modified live vaccines, especially those against viral diseases designed for use in domestic animals, may be extremely dangerous when applied to wild animals. Not only may they cause fatal disease but the vaccinated animals may shed live virus into the environment, which could then infect free-living populations. Furthermore, the efficacy of most vaccines manufactured for domestic livestock is completely unknown when they are applied to wild species. Some vaccines, licensed to protect a domestic animal after a single dose (e.g. killed rabies vaccine), may fail to provoke a strong and sustained antibody response when applied to wild species and may need to be repeated on a number of occasions.^{10, 11, 14}

It is recommended that where possible, animals should be vaccinated several weeks prior to the planned release in order that adverse reactions, such as vaccine-induced disease or immunosuppression (especially if live vaccine has been used), may be detected.

If there is any doubt as to the safety of a particular vaccine for wildlife and there is a choice between a live vaccine and a killed (inactivated) vaccine, the killed vaccine should always be used. In all cases, it would be wise to consult the vaccine manufacturers, local zoological facilities and also related organisations, such as the **American Association of Zoo Veterinarians (AAZV)**, the **American Association of Wildlife Veterinarians (AAWV)**, the appropriate International chapter of the **Wildlife Disease Association (WDA)**, the **Zoological Society of London (ZSL)**, the **World Association of Wildlife Veterinarians (WAWV)**, the **European Association of Zoo and Wildlife Veterinarians (EAZWV)**, the relevant reference laboratory (named by **OIE** or **WHO**) or the regional wildlife disease authority, for specific advice.

The protection of free-ranging wildlife from the enzootic and epizootic diseases of contiguous domestic livestock is best achieved by ensuring that the livestock is regularly vaccinated against these diseases.

Other procedures

Accurate identification of the species and subspecies, and if possible, population of origin, should always be made for all animals destined for translocation and release.

Whenever possible, whole blood, and blood in EDTA should be collected and the serum or plasma banked. Either liquid nitrogen, a -70 degrees Celsius ultra freezer or a -20 degrees Celsius freezer that is **not self-defrosting**, should be used to conserve sera. Such sera can provide an important resource for retrospective disease evaluation.

Whole blood for serum and blood in EDTA should be stored at +4 degrees Celsius, if collected for a Complete Blood Count (CBC), and processed within 24 hours. Blood smears are best made and air-dried as soon as possible after collection.

The quarantine period also presents an opportunity to identify permanently all unmarked animals when they are anaesthetised or chemically restrained (e.g. tattoo, ear notch, ear tag, microchip). In addition, whenever animals are restrained or immobilised, a complete physical examination, including measurement of body weight and dental examination, should be performed. Diagnostic specimens should also be collected and stored as above, at this time.

Veterinary medical records

Complete medical records should be kept and be available for all animals during the quarantine period. Animals that die during the quarantine period should have a necropsy performed under the supervision of a veterinarian and representative tissues submitted for histopathological examination, with other laboratory analyses (i.e. cultures) applied as indicated.

The results of all tests, both positive and negative, should be recorded. Thus a bank of baseline data will be developed.

Finally, the medical history and records of the human attendants, including the veterinarian, must be kept and the attendants must be regularly screened for infection with transmissible diseases (e.g. tuberculosis, chicken pox, mumps, influenza, hepatitis A and B) and be up to date with rabies vaccinations and tetanus toxoid.¹⁷

Ethical considerations^{12, 13}.

The quarantine, screening, transport and release of captive-bred or wild-caught animals is likely to cause a degree of stress, which could be significant in some cases. While translocation or re-introduction schemes can have potential benefits for the viability of wild populations, they may not be in the best interests of the welfare of the individuals involved. It is therefore important that :

1. the balance of the welfare costs and conservation benefits should be kept under review during translocation and re-introduction programmes,
2. no more animals are used than are judged to be necessary for the success of the scheme, and
3. procedures (transport, handling, quarantine, release etc) are designed as far as possible to minimise any harm or distress to the animals concerned.

Suitability for release. If the animal has been in a captive situation for a long time or if it is captive born and raised, a careful assessment of the animal's level of habituation, the presence of aversion and flight behaviours, and its ability to compete for food and space

in the wild should be made. It may not be advisable to release the animal if it appears to be irreversibly habituated to human beings. In order to minimise this problem, all handling for tourism purposes etc should be stopped at least 6 months prior to release.

The following are recommendations and suggestions for appropriate quarantine, health screening, treatment and vaccination procedures for the major animal groups likely to be the subjects of translocation and release into the wild. Whenever possible, national and international reference laboratories experienced with the species or with closely related taxa and the diseases concerned, should be consulted.

Treatment disclaimer

Many of the drugs and vaccines mentioned in this booklet are not registered for use in wild animals. It is possible that they may produce unexpected and undesirable reactions, even death.

It is therefore recommended that the veterinarian in charge of any health screening project for wild animals, prior to their release into the wild, should obtain and administer all drugs in accordance with the relevant national Veterinary and Poisons legislation.

The mention of specific drugs and vaccines and their manufacturers, by name, does not imply a recommendation by the publisher, editor or sponsors. This information is given merely to indicate what products are available and to suggest their source.

The following worksheet provides a suggested Checklist for Quarantine and Health Screening of wild animals prior to Translocation. Please read the Explanatory Notes at the end of the Worksheet before completing the form.

Quarantine and Health Screening Worksheet for Animal Movements

1. SPECIES TO BE MOVED:.....

2a. FROM:..... 2b. TO:.....

3. NUMBER OF ANIMALS:.....

4. ANIMAL ID

ID Number	ID TYPE	AGE	SEX	OUTCOME	COMMENTS

Attach additional sheet if needed.

4. ANIMAL MOVEMENT CATEGORY: Wild to wild
 Wild to captivity
 Captivity to wild
 Captivity to captivity

6a. PROJECT MANAGER:..... Tel:.....

6b. TITLE, INSTITUTION:..... Email:.....

7. PROJECT VETERINARIAN:..... Tel:.....
 Email:.....

- 8. DISEASES OF CONCERN** (relevant to source animals and destination animals including wildlife, domestic animals and humans). If more space needed, attach additional sheet to this form and include references

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9. SPECIFIC DIAGNOSTIC TESTS

(for documentation refer to individual animal records):

--

10. ROUTINE SCREENING/DIAGNOSTIC SAMPLES

(for results refer to individual animal records)

Diagnostic samples to be collected (check)	Collection dates	Date results received
Physical exam, body weight and measurements		
faeces		
Blood smear, haematocrit and total protein		
Whole blood, serum or plasma (max. volume / animal =)		
Fresh faecal or rectal swab for culture		
Choanal or oral swab for culture		
Ectoparasites		
Other:		

11. TREATMENTS/VACCINATIONS AND DATES

(for documentation refer to individual animal records):

--

12. SAMPLES TO BE FORWARDED TO:

.....

Quarantine Details

13. LOCATION:.....

14. FACILITY:.....

15. QUARANTINE DURATION: Begins..... Ends.....
Date Date

Total Days:.....
(If less than 30 days specify reason:
)

16. PERSON SUPERVISING QUARANTINE:

Tel:..... Email:.....

17a. TRAINING NEEDED FOR SUPERVISOR? Yes No

17b. DATE OF TRAINING, IF NEEDED:.....

18. QUARANTINE EQUIPMENT:

“Quarantine – no unauthorized entry” sign	Protective clothing	Feeding, watering and cleaning utensils
Insect/rodent traps/screens/baits	Cage furniture as appropriate for the species	Animal capture / restraint equipment
Diagnostic sample collection, storage and transport equipment	Animal record forms, pens	Quarantine register
Lock for facility	Bags for waste disposal	Animal caregiver health check
Footbath/boot changes	Other:	

19. BUDGET:

Personnel hours _____ @ _____	_____
Equipment costs	_____
Feed costs	_____
Lab costs	_____
Courier fees	_____
Veterinary fees	_____
Other (specify)	_____
TOTAL COST	_____

Budget Code _____

Movement Recommendation

- 20a.** Healthy and minimal threat to destination populations OK to move

- 20b.** Healthy but there is a significant threat to source animals Delay move
Cancel move

- 20c.** Unhealthy or threat to destination populations Delay move
Cancel move

Explanation and justification for recommendation:

21. FOLLOW UP ACTIONS:

22. PERMITS TO MOVE ANIMALS: YES NO

23. SIGNED OFF BY: _____ **DATE** _____
Project Manager

Quarantine and Health Screening Worksheet Explanatory Notes

1. **Species to be moved:** A separate sheet should be used for each species of animal moved.
- 2a. **From:** Point of origin of animals to be moved.
- 2b. **To:** Location to which animals will be moved.
3. **Number of animals:** Total number of animals to be moved
4. **Animal ID:** Each animal should be individually identified or a colony number assigned where individual identification is not possible. (e.g. amphibians or schools of fish):

Identification Number = number found on tag, tattoo, microchip, etc

ID type = tag, tattoo, etc.

Age = in years or where unknown classify as juvenile or adult

Sex = male, female, unknown

Outcome = Final disposition of each individual (e.g. moved, move delayed, canceled)

Comments = explanation of outcome

(Note “Outcome” and “Comments” in this table are inserted at the completion of the animal movement)

5. **Animal movement category:** Check the appropriate box
- 6a. **Project manager:** person responsible for the animal movement.
- 6b. **Title, Institution:** Project manager’s position in organization and name of organization
7. **Project veterinarian:** Designated veterinary advisor for this project.
8. **Diseases of Concern:** A complete list of diseases to which the species and its close relatives are susceptible should be compiled and evaluated for consideration of impact upon the animal movement. From this list those diseases which are likely to have a significant impact on source and/or destination populations are identified as “diseases of concern” for this animal movement.
9. **Specific Diagnostic Tests:** All specific diagnostic tests to be performed during quarantine, based upon diseases of concern (see number 8.)
10. **Routine Screening/Diagnostic Samples:** With assistance from the project’s veterinary advisor check the diagnostic samples to be collected and plan the date of collection. (Note that a physical exam should be performed on every individual animal). Additional diagnostic samples may need to be collected according to the needs of each diagnostic test.
11. **Treatments/vaccinations and dates:** List all planned treatments and vaccinations to be given during the quarantine period.

12. **Samples to be forwarded to:** List each laboratory's address and phone number. Make arrangements with laboratory prior to sampling. For some samples special handling and laboratories may be needed.

Quarantine Details

13. **Location:** geographic location of quarantine (e.g. zoo, park, island,)
14. **Facility:** specific building or site of quarantine.
15. **Quarantine duration:** **Begins** = the date the quarantine begins
 Ends = the last day of quarantine
 Total Days = total number of days of quarantine

Explain the length of quarantine chosen if it is less or greater than the standard 30 days. Animal husbandry issues (e.g. stress in captivity) may require that the quarantine period be shortened to less than 30 days; the length of time for test results to be received or a particular disease with a long incubation period may require that the quarantine period should be longer than 30 days.

16. **Person supervising Quarantine:** Name the individual responsible for maintaining animal health and quarantine status. Include his/her contact details.
17. a. **Training needed for supervisor?:** Indicate if the supervisor needs training in quarantine procedures and responsibilities (This may simply be a briefing).
17. b. **Date of training, If needed:** schedule date for training/briefing.
18. **Quarantine Equipment:** Check the appropriate boxes when the items listed have been organized.
19. **Budget:** identify costs associated with this animal movement project and include the appropriate budget codes(s).

Movement Recommendation

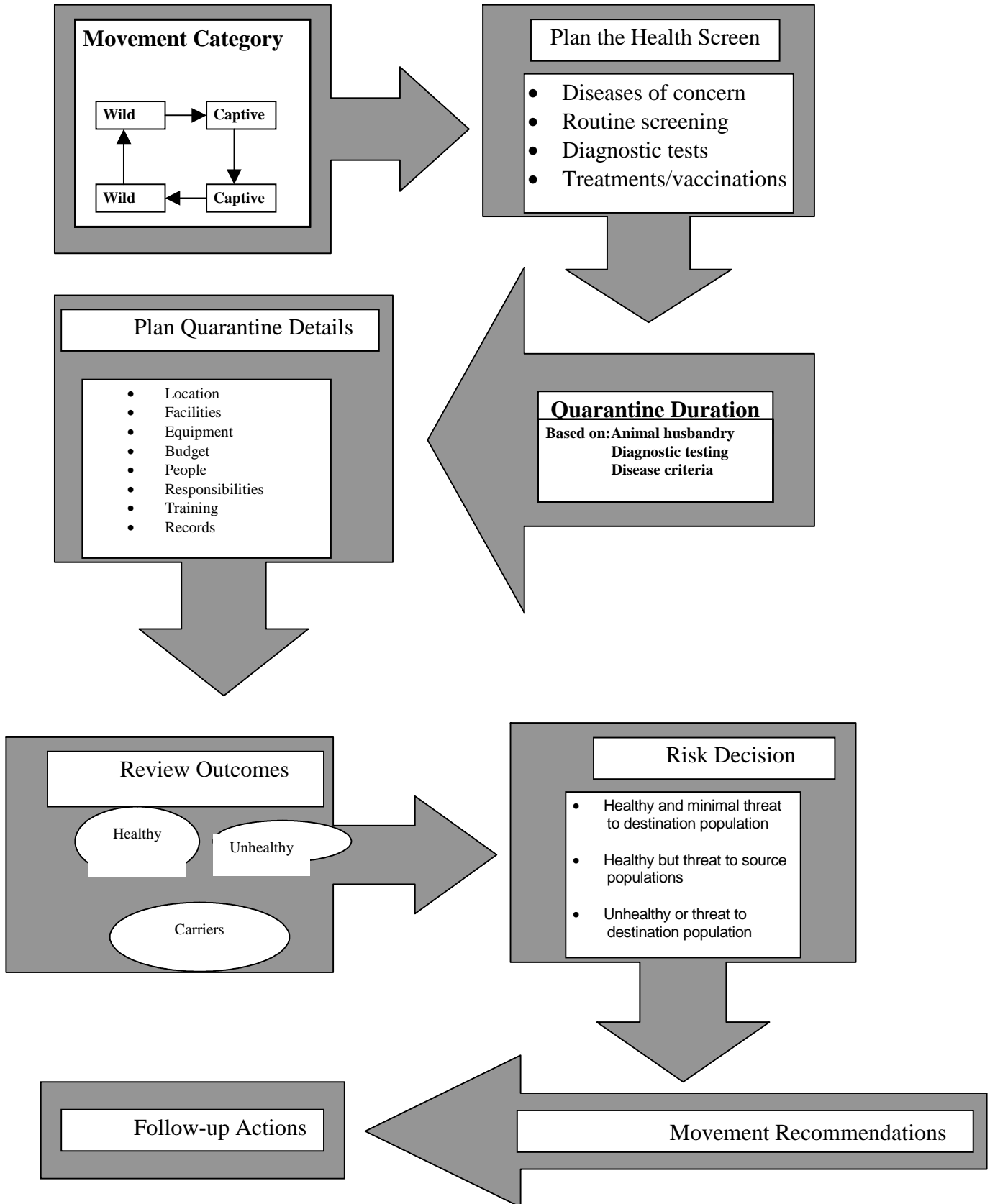
20. **a, b or c:** Check the appropriate box.

Use the accompanying box to explain and justify the decision made. Final recommendations to be based on results of health screen and disease considerations as listed in number 8.

21. **Follow up actions:** Indicate here any actions needed to follow through this animal movement. e.g., post movement surveillance of animals, review of animal movement procedures, etc.
22. **Permits to move animals:** Check the appropriate box to indicate if necessary permits to move the animals have been received.

23. **Signed off by:** At the completion of the animal movement project the Worksheet should be signed off and dated by the project manager.
21. This draft Quarantine and Health Screening Worksheet for Animal Movements has been written by Dr. Richard Jakob-Hoff, BVMS, Auckland Zoological Park, Grey Lynn, Auckland, New Zealand, and is reproduced here by kind permission of the New Zealand Department of Conservation.

Summary



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7. Jacobson, E.R., J.L. Behrer and J.L. Jarchow, (1999), Health assessment of Chelonians and release into the wild. *In: Fowler, M.E. and E.R. Miller (eds.), Zoo and Wild Animal Medicine: Current Therapy, 4*, chapter 30, pp. 232-242. W.B. Saunders & Co., Philadelphia.
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Gascoyne, S.C., Bennett, P.M., Kirkwood, J.K. and Hawkey, C.M. 1994. Guidelines for the interpretation of laboratory findings in birds and mammals with unknown reference ranges: plasma biochemistry. *The Veterinary Record*, **134**, 7-11

Gard, G., 1999. *Quarantine Requirements for Zoo Hoofstock*. A report for the Australian Quarantine and Inspection Service (AQIS), GPO Box 858 Canberra, ACT 2601, Australia.

The above is a very useful account of the important transmissible diseases that may affect **wild hoofstock** and may be transmitted to other animals in recipient institutions, to domestic or wild animals in the importing country or to humans, and of the risk management strategies to prevent their entry.

ARTIODACTYLA

Certification

An appropriate Zoo-Sanitary Certificate, duly signed by an Official Veterinarian of the exporting country, should accompany all imported animals destined for release into the wild. This certificate will contain information concerning any tests or vaccinations, which have been carried out prior to shipment. It may, however, be deemed advisable to repeat some or all of these tests after arrival during the quarantine period.

Ruminants imported from Foot and Mouth Disease (FMD) enzootic areas, especially from Africa, must be subjected to the "probang test" so as to exclude the presence of any FMD virus. The "probang test" samples must be examined at an approved FMD Reference Laboratory (Pirbright, UK or Onderstepoort, South Africa) and the results should be known before the animals leave the country of origin. Strict regulations govern the import of animals from FMD enzootic countries or areas, into FMD-free countries.

There are other potentially devastating diseases that also merit thorough attention as listed below.¹

Health screening while in quarantine

The suggested screening procedures, which follow, are necessarily lengthy. However, they may be modified and adapted depending on the genus and species of the translocated animals, their source (a zoo? a ranch? the wild?), their country or area of origin and destination, their period in captivity and their disease history. Interpretation of the results of the tests must also take the above information into account, along with knowledge of the epidemiological situation for the various diseases in the countries of origin and destination.

Tests, which give negative results (especially faecal examinations for endoparasites), may be repeated several times at suitable intervals. Where testing for exotic diseases is not possible locally, samples may have to be sent to an approved laboratory in another country. However, note that the shipment of diagnostic specimens may require various permits (including those from CITES) from the authorities in both the country of export and the country of import.

Quarantine for artiodactyla should be for a minimum of **30 days** (see **page ?** for special quarantine period to allow for requirements for testing for tuberculosis and brucellosis) during which the following procedures should be carried out:

1. Faecal examination (direct, flotation and sedimentation) for endoparasites, (especially *Fasciola magna*, which is best screened for by sedimentation procedures).
2. Baermann tests for lungworm larvae.
3. Faecal culture for Johne's disease and *Salmonella sp.*
(For *Salmonella sp.* culture it is recommended that multiple samples be taken over time and for Johne's disease it should be noted that conventional culture could take

12-18 weeks). However, radiometric mycobacterial culture techniques are much faster and can be completed in 6–8 weeks.

Note: ELISA and AGID (Agar Gel Immuno-diffusion test) are not validated for use in non-domestic hoofstock and in many instances have not produced satisfactory results. See “Proceedings of the Workshop on Diagnosis, Prevention, and Control of Johne’s Disease in Non-Domestic Livestock”, White Oak Conservation Center, 3823 Owens Road, Yulee, Florida 32097, U.S.A. July 26-28, 1998, pp. 63.

4. Examine blood smears for haemo-parasites. Carry out serology and if appropriate, xenodiagnosis, for important haemoparasites for those animals, which are negative on blood smear examination.
5. Complete Blood Count and Packed Cell Volume (PCV).
6. Examine the buffy coat of micro-haematocrit tube blood samples for trypanosomes and/or microfilariae.
7. Urinalysis when possible.
8. Serum/plasma chemistry profile (this may be expensive and unnecessary).
9. Serology for brucellosis (multiple tests are desirable for all exotic artiodactyla).³
10. Serology or PCR for malignant catarrhal fever (MCF), bovine virus diarrhoea (BVD), rinderpest (RP), peste des petits ruminants (PPR), lumpy skin disease (LSD), bluetongue (BT), epizootic haemorrhagic disease (EHD), Infectious bovine rhinotracheitis (IBR), leptospirosis, anaplasmosis.⁴
11. Virus isolation tests for bluetongue and epizootic haemorrhagic disease - these tests must be carried out during the pre-export quarantine (where applicable) and any animals found to be viraemic with BT must be rejected. Virus isolation tests for EHD are needed only for animals originating in USA. **Note:** Since ungulates that recover from rinderpest never re-excrete the virus, those that test serologically positive for rinderpest antibodies may be judged to be virus-free and safe to translocate after 30 days in quarantine.
12. Serological tests for tuberculosis (ELISA, Gamma-interferon etc.).⁵ Intradermal skin tests may be useful but must be carefully interpreted (e.g. false positives in caprines).

Advice on appropriate tests for tuberculosis in various species may be obtained from: **Central Veterinary Laboratory**, Lelystaad, Holland; **Central Veterinary Laboratory**, Weybridge, Surrey, UK; **Ministry of Agriculture Veterinary Laboratory**, Invermay, New Zealand; **Onderstepoort Veterinary Institute**, Pretoria, South Africa; **USDA/APHIS, AZA Animal Health Committee** and **CNEVA**, France.

See also “Tuberculosis Surveillance Plan for Non-Domestic Hoofstock” currently being developed by the National Tuberculosis Working Group for Zoo and Wildlife

Diseases, chairperson: Dr. S. Mikota, ACRES Species Survival Center, 140001 River Road, New Orleans, Louisiana 70131, USA.

13. Check for mycoplasmal kerato-conjunctivitis (especially in chamois (*Rupicapra* sp.) mouflon (*Ovis musimon*) and ibex (*Capra ibex*)). Some animals may be symptomless carriers and this infection can recur up to three months after recovery from clinical disease. Recovered animals should not therefore be moved into areas free from this disease.⁹
14. Check for ectoparasites (especially sarcoptic and psoroptic mange, warble fly larvae, nasal bot fly larvae, screwworms and ticks).
15. Ear mark, tag or insert microchip when possible.
16. Ultra-freeze suitably labelled serum and tissue samples.

Pre-release immunisations

As appropriate, any or all of the following immunisations may be made after taking into consideration the epidemiological situation in the country or region of origin and the eventual destination of the animals. In most cases a killed, inactivated vaccine is preferable to a living, attenuated product. While rinderpest vaccine will protect against PPR and vice versa, in most cases it is better to use PPR vaccine for small ruminants, so as to avoid confusion when later sero- sampling is carried out, especially in a country with RP-free status.

Anthrax (*Bacillus anthracis*). [Anthrax vaccine (Colorado Serum Company)].
 Pasteurellosis (*Pasteurella multocida*, Types B & E)
 Clostridial diseases (*Clostridium chauveii*, *Cl. septicum* and *Clostridium botulinum* (Types C & D). [Vision 7, (Bayer Corporation) or Heptavac (Hoechst Rousel Vet Ltd.)]
 Rinderpest
 Peste des petits ruminants
 Foot and Mouth Disease (Types A, O, C, & Asia 1)
 Rabies [Imrab 3, (Merial Ltd.)]
 Infectious bovine rhino-tracheitis (Many vaccines are available, always use a killed product such as Triangle 3, combined IBR, BVD, and PI3 (Fort Dodge Animal Health)]
 Bluetongue /Epizootic haemorrhagic disease
 Bovine virus diarrhoea (BVD). [See above]
 Contagious caprine pleuropneumonia (CCPP), (caprines only)
 East Coast Fever (ECF), (+ prophylactic antibiotic)
 Parainfluenza 3. [See above]
 Leptospira 5-way bacterin (Many vaccines are available, such as Leptoferm-5, (Pfizer Animal Health)]
 Paratuberculosis (Johne's Disease). Vaccine is now made in Spain for sheep

Note: Pre-release exposure to the vectors of protozoal haemoparasites may be advantageous, if carefully controlled. For example, if a pre-release enclosure is

located in low density tsetse fly (*Glossina sp.*) country, a low exposure to trypanosomiasis will occur and a useful immunity may result. However, if the animals are released without pre-exposure, they may choose a habitat with high tsetse fly challenge with subsequent disease risk.

South American camelidae should be routinely immunised with tetanus toxoid (most licensed products are satisfactory) and with a multi-valent clostridial toxoid such as Vision 7 with SPUR (Bayer Corporation) or Heptavac (Hoechst Rousel Vet Ltd.). However, there are many other satisfactory multivalent clostridial vaccines available on the market. Low volume, low reactivity products should be chosen because many clostridial vaccines often cause local reactions and abscesses.

Llamas are very sensitive to some anthrax vaccine strains.⁶

Other artiodactyla may be immunised against clostridial diseases according to local exposure risk and history.^{6, 7, 8}

Suidae/Tyassuidae may be immunised against leptospirosis with a multi-valent bacterin and against swine erysipelas. The need for these and other immunisations (e.g. Classical swine fever) should be based on local history and risk of exposure.

Note: In any situation where immunisation of wild artiodactyla is being considered, the local domestic animal disease regulatory authorities should be consulted. The effect of vaccine on the subsequent ability to detect disease by serology must also be considered. If a vaccinal titre cannot be distinguished from an exposure titre, the ability to monitor disease in the wild after release may be compromised, eg. rinderpest.

Pre-release treatment

1. All ruminants should receive two treatments, 14 days apart, with a recognised flukicide to control fascioliasis. In the USA, Albendazole, Valbazen (SmithKline Beecham Animal Health) or Clorsulon, Curatrem (Merck Agvet) can be used. In other countries these products may be available under different names.
2. All ruminants should be treated with an anthelmintic in the avermectin group (i.e., ivermectin (Ivomec, Merck Agvet), doramectin (Dectomax, Pfizer Animal Health), eprinomectin (Eprinex, Merial Ltd.), or moxidectin (Cydectin, Fort Dodge Animal Health)) at appropriate dosage during pre-import quarantine and again during post-import quarantine. Such treatment will control or eliminate endo-parasites and ecto-parasites (especially warble fly larvae and nasal bot fly larvae). Doramectin and Moxidectin have much longer lasting effect times than Ivermectin in ruminants.

Note: If mature warble fly larvae are manually squeezed out of the skin, great care must be taken to ensure that the larvae are not crushed, as this may precipitate a serious anaphylactic reaction. Avermectins, administered when the warble-fly larvae are approaching maturity under the skin, can cause a similar reaction).

3. Where necessary, administer trypanocidal treatment for trypanosomiasis.
4. Administer appropriate treatment for rickettsial diseases, if necessary.

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PERISSODACTYLA

These recommendations are based on those for domestic equidae (except where noted otherwise) which have been imported from another country. As appropriate, they can be adapted to cover wild equids, tapirs and rhinoceroses, which are destined for release into the wild.

Certification

An appropriate Zoo Sanitary Certificate, duly signed by an Official Veterinarian of the exporting country, should accompany all imported animals destined for release into the wild. This certificate will contain information concerning any tests or vaccinations, which have been carried out prior to shipment. It may be deemed advisable to repeat some or all of these tests after arrival, during the quarantine period.

Health screening while in quarantine

The suggested screening procedures that follow are necessarily lengthy. However, they may be modified and adapted depending on the genus and species of the translocated animals, their source (a zoo? a ranch? the wild?), their country of origin and destination, their period in captivity and their disease history. Interpretation of the results of the tests must also take the above information into account along with knowledge of the epidemiological situation for the various diseases in the areas or countries of origin and destination.¹

Where testing for exotic diseases is not possible locally, the samples may have to be sent to an approved laboratory in another country.

Quarantine for equids should be for a minimum of **30 days** during which the following procedures should be carried out:

1. Faecal examination (direct and flotation) for endoparasites.
2. Baermann tests for lungworm larvae (especially wild asses).
3. Submit a serum sample to an approved Reference Laboratory for testing for African horse sickness. (This should have been done during pre-export quarantine in the country of origin and any animals testing positive should not have been shipped).
4. Submit serum samples from each animal to an approved laboratory for the following tests:
 - a. Complement fixation test for dourine (*Trypanosoma equiperdum*).
 - b. Complement fixation test for glanders (*Pseudomonas mallei*).
 - c. Immunodiffusion (Coggins test) for equine infectious anaemia.
 - d. Serum neutralisation test for equine viral arteritis.
 - e. Test for equine herpes viruses (zebras and onagers)
 - f. Test for Potomac Horse Fever/Erlichiosis (*Erllichia risticii*), if from an enzootic area.
 All the above tests must prove negative.

*** Any animal which tests positive for any of the first four of the above diseases should be retested and if still positive, should be destroyed at once!**

5. Examine blood smears for haemo-parasites (*Babesia equi* and *B.caballi*, *Erllichia risticii*). Carry out serology on those that are negative on blood smear examination.

6. Examine the buffy coat of micro-haematocrit tube blood samples for trypanosomiasis (*Trypanosoma evansi*). Examine those negative for trypanosomes by ELISA test.
7. Test tapirs (*Tapirus* sp.) for bovine tuberculosis.
8. Complete Blood Count and PCV.
9. Serum/plasma chemistry profile.
10. Urinalysis if possible.
11. Check for ectoparasites.
12. Check for endoparasites.
13. Ear mark, tag or insert microchip when possible.
14. Ultra-freeze suitably labelled serum and tissue samples.

Pre-release immunisations

As appropriate, all or some of the following immunisations should be made after taking into consideration the local epidemiological situation and the eventual destination of the animals ²:

Tetanus [Many vaccines are available—Super-Tet (Bayer Corporation)]
 Rabies [Imrab 3 (Merial Ltd.)]
 Anthrax [Anthrax Vaccine (Colorado Serum Company)],
 Equine influenza [(Many vaccines are available – use a killed product such as Fluvac or Duvaxyn IET-Plus, which also contains tetanus toxoid (Fort Dodge Animal Health)]
 Equine herpes virus type -1 (rhino-pneumonitis) [Many vaccines are available –use a killed product, such as Prestige with Havlogen (Bayer Corporation)]
 Encephalo-myelitis
 Strangles
 Botulism
 Eastern equine encephalitis (EEE). Many vaccines are available –use a killed product, such as Encephaloid (Fort Dodge Animal Health)]
 Western equine encephalitis (WEE). [Encephaloid contains both EEE and WEE antigens].

Black rhinoceroses (*Diceros bicornis*) should be immunised against leptospirosis with a multivalent leptospirosis bacterin including *L. interrogans* var.*icterohaemorrhagiae* (after first checking leptospiral titres). The Black Rhino American Zoo and Aquarium Association (AZA) Species Survival Plan (SSP) recommends using Leptoferm-5 (Pfizer Animal Health).

Vaccination should be carried out annually. The use of BratiVac-6 (Pfizer Animal Health) for both black and white rhinos may be indicated because it has the *L. bratislava* serotype.added.

Note: Vaccination of black rhinos in captivity against leptospirosis has had significant side effects. These have ranged from sweating to severe sloughing of the skin. However, vaccination is still recommended. Check for tick infestation (possible vectors of cowdriosis-heartwater). If present, treat with an acaricide.

Pre-release treatment

1. As appropriate, suitable anthelmintics can be administered to remove or control endoparasites, especially liver flukes in tapirs.
2. Appropriate pyrethrin-type or pyrethroid acaricides should be applied, [Permethrin 11 (Boehringer Ingelheim Animal Health) may be used but there are many others] and any of the previously mentioned avermectins should be considered for the elimination of ectoparasites.
3. Appropriate treatment should be applied to eliminate any detected haemo-parasites.

References

1. Phillips, L.G. (1999), Infectious Diseases of Equids. *In*: Fowler, M.E. and E.R. Miller (eds.), Zoo and Wild Animal Medicine: Current Therapy, 4, chapter 84, pp. 572-574. W.B. Saunders & Co., Philadelphia.
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PRIMATES

The following are recommendations and suggestions for appropriate testing procedures for diseases of primates held in quarantine.

If primates have been held captive and in close contact with humans, care must be taken to screen them for all those diseases that are transmissible between human and non-human primates. Information about such diseases on a regional basis can be obtained from local medical authorities but it is important to remember that if foreign research workers or tourists have had access to the primates, exotic infections acquired overseas may occur in both the human and non-human primates.

The risk of zoonotic disease transmission, in both directions, is greatly reduced if all attendant staff always wears facemasks, gloves and gowns when in contact with primates.

Health screening while in quarantine

In view of the high risk of primates carrying zoonotic diseases, quarantine requirements are stringent. Primates should be held in strict quarantine for at least **30 days** after arrival and before any further relocation. Many institutions insist on **60 days** of quarantine and increase this to **90 days** for animals of unknown medical histories, those with known exposure to infectious disease, or for wild-caught animals.^{1, 2, 13}

1. Faecal examination (direct and flotation) for endoparasites, especially *Entamoeba sp.* which often infect primates, causing diarrhoea in animals subjected to stress. Since *Entamoeba sp.* are shed intermittently, several samples should be examined.

Primates should have three consecutive negative faecal examinations before release. Faecal samples from New World primates should be examined by sedimentation to increase the likelihood of detecting *Prosthenorchis sp.* ova that may be shed intermittently in small numbers. Where possible, a centrifugation technique should be substituted for the flotation procedure as this technique is more sensitive and will detect ova present in smaller numbers than standard flotation. Check for Spirurid nematodes (carried by cockroaches), especially in small South American primates, such as marmosets and tamarins.

2. Faecal culture for *Salmonella sp.*, *Shigella sp.* (Gorillas (*Gorilla sp.*) are particularly susceptible), *Campylobacter sp.* and *Yersinia sp.*

The need to carry out these tests should be based upon the history of the animals and their origin. Since these organisms are often shed intermittently by primates, which may exhibit no clinical signs of infection, three consecutive faecal cultures may be required.

3. Appropriate serology, based on history and origin, for toxoplasma, retroviruses, parainfluenza, measles, cytomegalovirus, Simian Immunodeficiency Virus (SIV) and Hepatitis A, B, C (HAV, HBV, and HCV). All Hepatitis virus infections in primates are diagnosed serologically. Chimpanzees (*Pan sp.*) are the only primate species, other than humans, that are susceptible to infection with all known human hepatitis viruses, but they rarely show any clinical signs.

Given that the prevalence of hepatitis virus infections among the general human population is high, especially in areas where faecal contamination of food and water supplies is frequent, it is not uncommon to find primates that have already been exposed to these viruses by their human caretakers and local contacts.¹¹

Although there is little evidence that HBV infection has been transmitted from primates to humans, in view of the potential health risk for contiguous human communities, non-human primates serologically positive for HAV, HBV or HCV (or indeed any other major zoonosis), should not be released into the wild.

Since it appears that HBV infection can occur naturally, without any clinical signs, in some chimpanzee populations,¹² it may be justified to release HBV-positive chimpanzees into wild populations, when these are already known to be infected by this virus and are suitably isolated from human settlements.¹⁰

Personnel working with non-human primates should consider vaccination for both HAV and HBV.

All Old World primates, including the Great Apes (*Gorilla sp.*, *Pan sp.* and *Pongo sp.*), should be serologically tested for *Herpesvirus simiae* (Herpes B).⁸

Note: That the quarantine period for Herpes B infection in Old World primates is **42 days**. Some Macaque species (*Macaca sp.*) may shed this virus intermittently and may be seronegative, latent carriers of Herpes B virus. Due to the lethal potential of Herpes B virus for other primates, **including humans**, precautions must be taken to prevent exposure. Macaques from the Philippines should be tested for Ebola-Reston virus (a strain of Ebola virus) infection.

4. Carry out serum/plasma chemistry profile.
5. Carry out urinalysis if possible.
6. Carry out complete Blood Count and PCV.
7. Blood smears should be examined for *Filaria sp.* and *Plasmodium sp.* (malarial parasites). Filarial Infections, however, seem not to be pathogenic.
8. Three negative tests for tuberculosis should be performed at two-week intervals using a tuberculin containing at least 1500 units/0.1 ml (eg. Mammalian human isolate, Coopers Animal Health, Kansas City, Kansas, USA) or "old mammalian tuberculin" (Pasteur Institute, Paris, France). The tuberculin should be injected into the upper eyelid.

Note: Orang-utans (*Pongo pygmaeus*) and chimpanzees (*Pan troglodytes*) frequently exhibit false positive reactions to intradermal tuberculin testing and further diagnostic evaluation may be necessary to determine their true status.^{3, 5}

9. Carry out chest radiography.
10. Check for ectoparasites.
11. Apply indelible tattoo or microchip for identification. Full-face photographs are a good way to identify apes. All are different and can be used for recognition of individual apes, even in the field after release.
12. Ultra-freeze suitably labelled serum and tissue samples. At least 10 body hairs, with hair follicles attached, should be collected for later DNA analysis. These can be stored in dry envelopes and may be valuable when it is

desirable to check for genetic origin before release, in order to avoid “genetic pollution” of a recipient wild primate population. Use plastic surgical gloves and heat-sterilised forceps when collecting the hairs.

Pre-release immunisations

Primates, especially those likely to be exposed to humans when “habituated” for tourism, should be appropriately vaccinated.^{4,6}

1. **Hepatitis:** Chimpanzees can be vaccinated against hepatitis but if they are, it will be difficult to assess their serological status later. It is thus not advisable to use hepatitis vaccine if the apes are likely to be in close contact with humans after release.
2. **Poliomyelitis:** Oral vaccine should be administered to the Great Apes at three, six and nine months and at two years of age for young and juvenile apes. Adults of unknown history should receive oral polio vaccine three times at two months intervals.

Note: The use of oral polio vaccine in primates is somewhat controversial as live virus is shed in faeces when this is given. For this reason, parenteral polio vaccine (which is a killed product) is a better choice.

3. **Mumps:** Infection with this virus can cause orchitis and subsequent sterility. Vaccination may be advisable if contact with humans is likely.
4. **Measles:** Due to the high mortality that this virus can cause in some species (eg. colobus (*Colobus sp.*), silver leaf monkeys (*Presbytis sp.*) and New World monkeys), vaccination is recommended, particularly for those primates likely to come into contact with humans. Newly wild-caught monkeys are especially at risk and should be vaccinated. Juvenile, captive bred monkeys born to immunised females, may have prolonged maternal antibody protection and should be vaccinated at six months of age, with a booster at one-year-old. In the Great Apes, maternal immunity may last until the infant is 15 months old. Vaccination can thus be delayed until after that time. Human measles vaccine can be used but may cause transient immunosuppression or vaccine induced measles if animals are stressed soon after vaccination. It is therefore recommended that the stresses of tuberculin testing or shipment should be avoided soon after vaccination.
5. **Rabies:** Primates, like all mammals, are susceptible to infection with rabies virus. Vaccination in rabies-enzootic areas is recommended. A killed, inactivated vaccine, as recommended for human use, can be used but is expensive. Imrab 3 (Merial Ltd.) has also been used.
6. **Tetanus:** All primates are susceptible to tetanus and should be vaccinated before release. Standard human tetanus toxoid is suitable. A combination human vaccine containing diphtheria, pertussis (whooping cough) and tetanus (DPT) is sometimes used but since non-human primates are not susceptible to diphtheria or pertussis the use of this combination product is unnecessary.

Pre-release treatment

1. Appropriate anthelmintics should be administered to eliminate or control endoparasites.
2. Ectoparasites should be eliminated before release by applying pyrethrin-based acaricides and by administering an avermectin. Sarcoptic mange will require two consecutive treatments with an avermectin, followed by a negative skin scraping. Ivermectin (the porcine form) should always be injected subcutaneously. Abscesses and skin ulceration have been seen in chimpanzees after the use of the bovine form of "Ivomec".
2. Young apes, forcibly taken from their parents, are very sensitive to emotional stress and frequently die in captivity, particularly if they have been badly treated by their captors. A delicate balance must be found between the requirements of veterinary quarantine and the emotional needs of the orphans, which need continuous care and sympathetic attention if they are to survive.

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CARNIVORA

For marine carnivores, see under **Marine Mammalia**.

Health screening while in quarantine

Quarantine for carnivores should be for **30 days** unless rabies is a problem in which case quarantine may be increased to six months.

Note: Rabies incubation period is highly unpredictable and in some species may greatly exceed six months.

1. Faecal examination (direct, flotation, sedimentation and Baerman technique) for endoparasites.

Note: Intestinal ascarids of the genus *Baylisascaris* may occur in procyonids, mustelids and ursids in North America. These ascarids are rarely important to their definitive hosts but after the eggs of the nematode are shed in the host's faeces, the resultant larvae are neurotropic and viscerotropic in small mammals, birds and occasionally, humans. Wildlife that are normal hosts for this parasite are asymptomatic carriers; other wild species can become victims of larval invasion and the introduction of *Baylisascaris sp.* into a new environment could adversely affect wild rodents, lagomorphs and birds in which larval migration causes blindness, ataxia and paralysis. Faecal contamination by *Baylisascaris* infected animals has caused mortality in domestic poultry and two fatal human cases have been reported.

Note: Wolves (*Canis lupus*), foxes (*Vulpes sp.*, *Alopex sp.*, *Urocyon sp.*), coyotes (*Canis latrans*), jackals (*Canis sp.*), hyaenas (*Crocuta crocuta* and *Hyaena sp.*) and wild felids, must be carefully examined for infection with the tapeworms of *Echinococcus sp.* and even if the presence of these very small cestodes is not confirmed, all these animals should be treated with intramuscularly injected praziquantil [Droncit, (Haver-Lockhart, USA) and Bayer in Europe)]. Praziquantil does not kill the eggs of *Echinococcus sp.* and the faeces and hair of infected carnivores are heavily contaminated, thus presenting a very serious hazard to the human attendants and to the environment.

Rodents are the intermediate hosts for *E. multilocularis* and ungulates (wild and domestic) for *E. granulosus*. However, since the survival of the adult cestodes within the gut of the final host is relatively short (1-2 months), a period of quarantine, during which the carnivores are fed on cyst-free food, should remove the worms. **Both species of Echinococcus are dangerous and potentially lethal zoonoses.**

2. Check for ectoparasites, especially sarcoptic mange.
3. **Serology for Felidae:** Exposure to Feline immunodeficiency virus (FIV) ⁶ should be determined using Western blots, IFAs and ELISAs. Subpopulations of some species of large felids exhibit a wide range of lentivirus prevalence, with some subpopulations showing no evidence of infection. There are different specificities and sensitivities among lentivirus assays being applied to non-domestic felids, suggesting that **assay choice** is important and that caution is warranted in comparing and interpreting data.¹

To determine infection with feline leukemia virus (FeLV), an ELISA test for the presence of antigen should be used. To test for exposure to FeLV, an ELISA to test for the presence of antibody should be used.

Note: No non-domestic felid that is FeLV antigen positive should be released into the wild as this virus does not appear to be enzootic in either wild or captive felid populations.

To date, FeLV antigen positive non-domestic felids appear to have acquired the infection from domestic or feral cats.

Other infectious diseases of Felids to check for include: feline viral rhinotracheitis, calicivirus, panleucopaenia, coronavirus/ feline infectious peritonitis and toxoplasmosis (a zoonosis). Remember that previous vaccination will influence interpretation of the results.

For herpes virus, calicivirus and toxoplasmosis infections, a carrier state is probable. The risk of release into the wild of carrier felids depends on the risk to other susceptible indigenous wild felids (eg. the release of lynx (*Felis lynx*) into an area with a resident population of wild cats (*Felis sylvestris*) might be unwise.

4. Felids, canids and pinnipeds are all susceptible to heartworm disease and should be tested for *Dirofilaria immitis* by assays which detect cuticular antigen. Other carnivores in heartworm enzootic areas may also be tested.
5. **Serology for Canidae:** Carry out serology for the following diseases: canine distemper, adenovirus, parainfluenza virus, parvovirus and leptospirosis. Previous vaccination will influence the interpretation of the results.

For all Carnivora:

6. Carry out complete Blood Count and PCV.
7. Carry out serum/plasma chemistry profile.
8. Carry out urinalysis if possible. Examine for kidney worm eggs, especially in Maned wolf (*Chrysocyon brachyurus*) and for *Capillaria sp.*
9. Apply tag, tattoo or microchip for identification.
10. Deep-freeze suitably labelled serum and tissue samples.

Pre-release immunisations

Felidae

1. **Wild Felidae** should be routinely vaccinated with an inactivated vaccine derived from feline rhinotracheitis, feline calicivirus, feline *Chlamydia psittaci* and feline enteritis (panleukopaenia). [Fel-O-Vax PCT or IV (Fort Dodge Animal Health) are satisfactory]. Vaccination should be repeated at two-week intervals for three injections or until 16 weeks of age, then annually, when possible. Check titres, if concerned.
2. **Canine distemper** virus (CDV) has been implicated in recent deaths of lions that showed clinical signs of distemper, on the Serengeti Plains in Tanzania. Similar clinical findings were reported in the lions of the Masai Mar in Kenya. Adult orphan lions in captivity were subsequently vaccinated with live attenuated Onderstepoort strain of CDV. The results indicated that this vaccine is both safe and immunogenic for lions in high risk situations.⁸
3. **Rabies:** All felids are susceptible. Use killed vaccine. [Imrab 3, (Merial Ltd.)]. See possible legal constraint above.

Note: Check with local authorities. In some areas vaccination even with a killed rabies vaccine is illegal.

Canidae

1. **Canine distemper:** Vaccination with new commercial recombinant vaccine is recommended for all wild canidae. No killed vaccine is commercially available at present. The modified live virus vaccine of canine cell line origin has been associated with vaccine-induced distemper in wild canids. However, modified live virus primate cell line products have provided protective titres and have not been associated with vaccine-induced disease in wild canids.⁷
Galaxy D (Schering-Plough Animal Health Corporation) appears to be the safest modified live commercially available at present.
2. **Canine infectious hepatitis:** Vaccination with canine adenovirus type 2, modified live virus vaccine is available and can be used. There may be some risk of hepatitis in maned wolves. The diluent portion of Adenomune-7 (Biocor, Pharmacia and Upjohn) has a killed adenovirus type 2 antigen that can be used for maned wolves.
3. **Leptospirosis:** Biannual vaccination with a multivalent bacterin is recommended. The diluent fraction of many canine vaccines contains killed bacterins for leptospirosis. The diluent fraction of Adenomune-7 (Biocor, Pharmacia & Upjohn) can be used or Nobivacc Lepto (Intervet U.K. Ltd.) is also available.
4. **Canine parvovirus:** All exotic canids should be considered susceptible. Bush dogs (*Speothos veraticus*), have developed parvovirus enteritis even when vaccinated under a regular canine regime and repeated vaccination is recommended until protective titres develop. A suitable regime might be the use of a killed vaccine such as Parvocine (Biocor, Pharmacia and Upjohn) for pups starting at 6-7 weeks old and then vaccinating every 3 weeks until 16 weeks of age; pups should then be sero-sampled and if satisfactory titres are present, they should be given a modified live vaccine (MLV), such as Duramune KF-11 (Fort Dodge Animal Health) at 6 months of age and then annually. In high contact areas where parvovirus is enzootic, vaccination every 6 months may be advisable.
5. **Parainfluenza** vaccination is recommended.
6. **Rabies** vaccination using a killed vaccine is always recommended [Imrab 3 (Rhone Merieux, Inc.)]. If a killed rabies vaccine, licensed to protect domestic dogs after a single dose, is used, the antigenic response should be checked and if found to be inadequate, the vaccination may need to be repeated several times at suitable intervals until protective antibody titres are found to be established.

Note: Check with local authorities. In some areas vaccination, even with a killed vaccine, is illegal.

There is a need for further testing of rabies vaccination protocols and delivery systems before vaccination against rabies can be relied upon to be fully protective for wild animals.^{2,3}

Procyonidae [Raccoons (*Procyon lotor*), coatimundi (*Nasua nasua*), kinkajou (*Potos flavus*), red panda (*Ailurus fulgens*)].

1. **Canine distemper.** All are susceptible. Annual vaccination is recommended with modified live primate cell line vaccine. Galaxy D (Shering-Plough Animal Health) is probably the safest but a canary pox-vectored vaccine would be safer, if available for all species other than red pandas and giant pandas (*Ailuropoda melanoleuca*), which should be vaccinated only with a killed vaccine. But be aware that this may not provoke a solid immunity.⁷
2. **Feline panleukopaenia** (or at least a parvovirus) has been reported in raccoons and coatimundis. Vaccination with a killed vaccine such as Fel-O-Vac PCT (Fort Dodge Animal Health) is recommended as it produces good FPV titers).
3. **Leptospirosis** is reported in raccoons. Biannual vaccination with a multivalent bactrin is recommended [Kavak-L (Fort Dodge Animal Health), or Nobivacc Lepto (Intervet, U.K. Ltd.)]
4. **Rabies** affects all species. Vaccinate with a killed vaccine. [Imrab 3. (Merial Ltd.)] See possible legal constraint above.

Mustelidae (Mink, ferrets (*Mustela sp.*), weasels (*Mustela sp.*), otters (*Lutra sp.*), sea otters (*Enhydra sp.*), skunks (*Mephitis sp.*) etc.).^{4,5}

1. **Canine distemper:** All members of this group are susceptible, some extremely so. All should be vaccinated with a product **NOT** of ferret or canine cell line origin. Vaccines of some avian cell lines have been shown to be safe and effective. However, since avian cell line products, when administered to red pandas and viverrids as part of a multivaccine have resulted in disease, possibly due to immunosuppression and anaphylaxis, it is recommended that the canine distemper vaccine be given separately at a reasonable interval from the other components.

A monovalent, recombinant canine distemper vaccine, canary pox-vectored, for use in ferrets, is under development by Merial, Ltd but is as yet unlicensed. This vaccine has undergone some preliminary clinical trials and has been found to be safe and effective in a variety of wild carnivores, including some felid species.⁷

Galaxy D (Shering-Plough Animal Health) can be used in some mustelids and viverrids; however, it must be used with extreme caution in these species as vaccine-associated disease can occur. If unsure, always use killed CDV vaccine or canary pox-vectored vaccine. Canine distemper vaccines, which are safe for wild carnivores are currently under development and it is wise to consult the manufacturers.

2. **Feline panleucopaenia:** All except the domestic ferret (*Mustela putorius*) are susceptible. Vaccinate with killed vaccine such as domestic cat vaccine, without respiratory virus components. When indicated, use Fel-O-Vac PCT (Fort Dodge Animal Health).
3. **Rabies:** All are susceptible. A killed virus vaccine [Imrab 3 (Merial Ltd.)] has been tested and found to be safe and efficacious and has been approved for use in domestic ferrets by FDA. See possible legal constraint above.

4. **Botulism:** Commercially raised mink are routinely vaccinated with toxoid against *Clostridium botulinum* type C. This may be unnecessary for wild mustelids prior to release if the animals are fed appropriately during quarantine.
5. **Leptospirosis:** Has been reported in mustelids. If considered a risk, vaccination with a multivalent bacterin is recommended [Kavak-L (Fort Dodge Animal Health) or Nobivacc Lepto (Intervet, U.K.Ltd)].

Viverridae [Civets, mongooses, meerkats (*Suricata suricatta*) and binturongs (Arctictis binturong)].

1. **Canine distemper:** All are susceptible and vaccination with avian cell line modified live vaccine may be carried out. However, avian cell line vaccines are no longer available in the USA. Galaxy D vaccine (Shering Plough Animal Health) can be used in some cases but care must be taken. It is unsafe for binturongs. If unsure, use a killed vaccine alone, **Not** as part of a multivalent vaccine (see above). Binturongs may even develop vaccine-induced disease with avian cell line vaccines, therefore consider using killed or canary pox-vectored for this species.
2. **Feline panleucopaenia:** All viverrids should be vaccinated. Use Fel-O-Vac PCT (Fort Dodge Animal Health) when indicated.
3. **Leptospirosis:** has been reported in mongooses and vaccination with multi-valent bacterin is recommended [Kavak-L (Fort Dodge Animal Health) or Nobivacc Lepto (Intervet, U.K. Ltd.)].
4. **Rabies:** All are susceptible and should be vaccinated with a killed vaccine [Imrab 3 (Merial Ltd.)].

Hyaenidae

1. **Canine distemper:** Vaccination with a killed vaccine is recommended but some authorities believe that hyaenas are not susceptible to infections with canine distemper.
2. **Feline panleucopaenia:** Susceptibility is uncertain but vaccination is recommended. If indicated, use Fel-O-Vac PCT (Fort Dodge Animal Health).
3. **Rabies:** All are susceptible. Vaccination with killed virus vaccine is recommended [Imrab 3, (Merial Ltd.)]. . See possible legal constraint above.

Ursidae

1. **Canine infectious hepatitis:** Naturally occurring disease has been reported once in black bears (*Ursus americanus*). Vaccination should be considered when

translocated bears might come into contact with domestic dogs. Consider using the diluent fraction of Adenomune-7 (Biocor, Pharmacia & Upjohn).

2. **Canine distemper** vaccine (killed) and feline panleucopaenia vaccine may be required for polar bears (*Ursus maritimus*).
3. **Rabies:** Bears are susceptible and should be vaccinated using killed virus vaccine [Imrab 3 (Merial Ltd.)] when rabies presents a risk.

Pre-release treatment

1. Appropriate anthelmintics should be administered to eliminate or control endoparasites.
2. Ectoparasites should be removed by applying pyrethrin-based acaricides and/or by administering an avermectin when necessary. For sarcoptic mange, two administrations of an avermectin are required, followed by a negative skin scraping. Fipronil, Frontline Top Spot and Spray (Merial Ltd.) and Imidacloprid and Advantage (Bayer Corp.) are being used with great success in many exotic carnivore species for ectoparasite control (especially for fleas).

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MARINE MAMMALIA^{4, 5, 6}

Marine mammals are sometimes found stranded on the beach, singly or in groups. It will probably not be possible to quarantine the animals in these circumstances but a limited health screen before return to the ocean is desirable.

Quarantine for marine mammals should last for a minimum of **30 days**.

However, this period is somewhat arbitrary because knowledge of disease and disease pathogenesis in marine mammals is incomplete.

Health screening while in quarantine

Veterinary medical history should include the following: Site of stranding, health or condition at stranding if known, disease history (current, clinical and serologic), treatment received during rehabilitation, documentation of physical and behavioural history and exposure to disease.

If an animal is held with or in close proximity to other animals undergoing rehabilitation, the disease history of pen-mates and neighbours should be considered to ensure that new diseases have not been contracted while in rehabilitation.

Animals should be free of any therapeutic drugs for a minimum of one week prior to release into the wild. This is to prevent drugs masking the signs of disease and to minimise the development of drug-resistant organisms.

1. Carry out faecal examination (direct smear, flotation and sedimentation) for endoparasites. Both sedimentation and flotation are required to detect *Otostrongylus circumlitus*.

A concentration heartworm test (Knott's procedure or Filter test) should be carried out to screen for microfilaria. If microfilaria are seen they should be identified down to species. A large proportion of *Zalophus sp.* (Californian sea lions) and *Callorhinus sp.* (fur seals) may be positive for microfilaria of the inconsequential fascial worm.

2. Carry out complete Blood Count and PCV.

3. Carry out serum/plasma profile.
4. Carry out urinalysis if possible.
5. Carry out serology and/or PCR for the following infections of **Pinnipeds**: Morbillivirus, herpesvirus, influenza virus, canine parvovirus, calicivirus, leptospirosis, brucellosis and dirofilaria. For **Cetaceans**: Morbillivirus, herpesvirus, and brucellosis. Serology for morbilliviruses should include canine distemper virus, phocine distemper virus, dolphin morbillivirus and porpoise morbillivirus).^{1, 2, 3}
For **sea otters** (*Enhydra lutra*): Test for brucellosis, tuberculosis, and pseudotuberculosis.
6. Apply tag, tattoo or microchip for identification.
7. Ultra-freeze suitably labelled serum and tissue samples.

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RODENTIA and LAGOMORPHA^{3, 4} (Compiled by A.W. Sainsbury¹)

The translocation of Lagomorphs (and their products) has proved to be a hazardous procedure. The example of myxomatosis has shown that the spread of a virus that is benign

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for its host species, the South American Forest Rabbit or Tapeti (*Sylvilagus braziliensis*), can be virulent for another species, the European rabbit (*Oryctolagus cuniculus*), and can cause disastrous mortality in wild and captive populations, worldwide. Rabbit Viral Haemorrhagic Disease (RVHD), and European Brown Hare Syndrome (EBHS) are also examples of emerging virus diseases of obscure origin.

Bacterial diseases with possible zoonotic consequences and which are geographically localised include brucellosis (*Brucella suis* biovar 2) and tularaemia (*Francisella tularensis* biovar *tularensis* in North America and *F. tularensis* biovar *palaeartica* in northern Eurasia).

In countries that are free from these diseases, translocated rodents and lagomorphs should undergo quarantine and shown to be serologically negative before release.^{1,2}

Health screening while in quarantine

Quarantine for rodents and lagomorphs (hares and rabbits) should be for a minimum of **35** days during which the following procedures should be carried out.

1. Faecal examination (direct and flotation) for endo-parasites (three samples at 15 day intervals are recommended). Rodents and lagomorphs should ideally have three consecutive negative faecal examinations before release.
2. Faecal culture for *Salmonella sp.*, *Campylobacter sp.*, *Yersinia pseudotuberculosis*, *Clostridium piliforme* (three samples over ten days are recommended).
3. Bacteriological culture of the upper respiratory tract from a nasal swab.
4. Serological test for *Encephalitozoon cuniculi* in rabbits. Consider isolation and do not release animals that are positive for antibody. Serology for *Brucella suis* in European hares. Do not release animals positive for *Br. suis* antibodies. Consider risk of tularaemia (*Francisella tularensis*) for all lagomorphs and North American and northern Eurasian rodents. since tularaemia can be subclinical in these animals. Details of tests to be performed are found in the OIE Manual of Standards for Diagnostic Tests and Vaccines.
5. For squirrels (Sciuridae), serology for parapoxvirus based on their history and origin. Only squirrels serologically positive for antibody should be released in areas where parapoxvirus occurs.
6. Haematological analysis including blood count and PCV, if feasible. The volume of blood that it is possible to collect will be dependent on body size.
7. Serum/plasma chemistry profile, if possible.
8. Examine blood smears for haemoparasites.
9. Urinalysis whenever possible.
10. Check for ectoparasites.

11. Examine the incisor and molar teeth for abnormalities.
12. Carry out abdominal palpation for impaction and lesions of chronic infectious origin, such as amyloidosis.
13. Evaluate body condition.
14. Identify using a microchip or with an eartag (preferably both). Eartags should be placed in the proximal part of the ear and should be non-irritant. Toe-clipping is not recommended.

Pre-release immunisations

1. **Rabies.** If the animals are to be released in an area where rabies occurs vaccination with a killed rabies vaccine [Imrab 3 (Merial Ltd.) is recommended.
2. If lagomorphs are to be released where **Myxomatosis**, **Rabbit Viral haemorrhagic disease (RVHD, Calicivirus** infection of rabbits) and/or **European Brown Hare Syndrome (EBHS)** are prevalent, consider administering an appropriate vaccine. Alternatively, test for protective antibodies. Vaccine prepared for use in rabbits against RVHD may also be protective against EBHS.

Pre-release treatment

1. Administer appropriate anthelmintics or other parasiticides to remove or control endoparasites.
2. Apply appropriate pyrethrin-type or pyrethroid acaricides and an avermectin for the elimination of ectoparasites to reduce the possibility of tularaemia (*Francisella tularensis* infection) or myxomatosis in rabbits.
3. Consider the administration of antibiotics to reduce the severity of upper-respiratory tract infections based on nasal culture and sensitivity testing. Tetracyclines may be administered to decrease the severity of *Mycoplasma pulmonis* infection.
4. Many rodent species are carriers of *Leptospira sp.* and may serve as sources of infection for other animals and humans. The risk of transmitting leptospirosis to other animals at the release site should be considered.

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MARSUPIALIA

(Compiled by S.A. Haigh²)

Phascolarctidae (koalas)

Health screening while in quarantine

The quarantine period should be **60 days**. Koalas should be kept individually in isolation within insect and pest proof enclosures. If possible small groups to be released together should be kept together in quarantine. Enclosures and equipment should be disinfected with Parvocide (gluteraldehyde 100g/L and dimethyl benzyl ammonium chloride 150g/L, Cyanamid Websters) at 1%, or Halasept (chloramine 100%, Intervet Australia) at 0.3 %.

Health screening

Koalas should be given a complete physical examination at the beginning and end of the quarantine period including weight, normal clinical parameters, lymph node checks, abdominal palpation, tooth wear evaluation, condition score, checks for conjunctivitis, nasal and ocular discharges, fur staining around rump. Feet and hand pads should be checked for sores. Faecal pellets should be normal size, shape and consistency, and should contain fine particles. The pouch and cloaca should be checked, and in males the penis should be everted from the cloaca and checked. The ear pinnae and canals should be checked for wounds/infection (especially males). Any skin lesions should be scraped for ectoparasite checks and submitted for fungal culture.

Koalas being released into *Chlamydia*-free populations should have two negative ELISA tests¹ or complement fixation tests (CFT) for *Chlamydia* antibody² one month and at least two months apart respectively. If positive there should be no rise in titre. For the CFT a titre of 1:4 is considered negative. Each animal should have three consecutive negative swab tests by PCR/hybridisation detection.³ Two swabs should be taken each time; one from both eyes and both nostrils and one from urogenital sinus or penile urethra. The last test should be done just prior to release. If PCR is not available, a commercial antigen

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detection kit such as Clearview Chlamydia (Unipath Limited, Bedford, England) can be used. The receiving colony should have a history of regular negative antibody tests on serum or antigen tests on swabs.

All koalas should have full blood counts and biochemistry done on entry into quarantine and just prior to release. Azotaemia may indicate nephrosis, which is a relatively common disease of captive koalas. Haematologic abnormalities may indicate retroviral infection and/or myeloid neoplasia. All koalas should have at least one detailed urinalysis including examination for calcium oxalate crystals.

There should be three consecutive, negative faecal flotations for endoparasites. Treat with praziquantel at 5mg/kg twice (at beginning and end of quarantine) for the cestode *Bertiella obesa*. Reduce access to soil ingestion. Faeces should be submitted for complete bacteriologic examination including routine culture, anaerobic culture, *Salmonella* culture, *Campylobacter* culture and Ziehl Neelsen staining for acid-fast bacilli.

Test for *Cryptococcus spp.* infection- use Latex Cryptococcal Antigen Test (LCAT)⁴ on serum. Greater than 1:2 is considered positive. Two negative tests or non-rising titres are needed.

Test both the recipient population and departing koalas for retrovirus infections with PCR-based tests⁵ if possible. Many captive koalas are positive and it is suspected that this virus is intrinsic within the koala genome. Release positive animals only into a positive population.

Test the recipient and departing population for *Toxoplasma gondii* infection using a modified direct agglutination test for antibodies⁶. Release only negative animals into negative populations. A large proportion of positive animals in a wild population may indicate historic exposure to cat faeces.

Koalas may be tested for *Leptospira interrogans* antibodies using a microscopic agglutination test⁷. Koalas released from captivity or moved from non-paralysis tick (*Ixodes holocyclus*) enzootic areas should be released into tick free areas.

Pre-release immunisations

Koalas should probably be vaccinated against *Bordetella bronchiseptica* using an inactivated cell free extract of *B. bronchiseptica* (Canvac BB, Commonwealth Serum Laboratories Ltd.) subcutaneously, twice four weeks apart, then annually. Rhinitis/pneumonia complex is probably more relevant in captive koalas.

Suitability for release

If the animal has been in a captive situation for a long time or if it is captive born, a careful assessment of the animal's level of habituation, presence of aversion and flight behaviours, and ability to compete for food and space should be made. It may not be advisable to release the animal if it appears to be irreversibly habituated. All handling for

tourism purposes etc should be stopped at least 6 months prior to release. All koalas should be fully weaned and be 16-18 months of age prior to release or shipment to other institutions.

Vombatidae (wombats)

Health screening while in quarantine

The quarantine period for wombats is **60 days**.

Serology for herpes virus, *Leptospira spp*, *Toxoplasma gondii* antibodies (methodology as described above) should be done. Any skin lesion should be examined carefully for the presence of *Sarcoptes scabiei* mites. Even if mites are not seen, and there are skin lesions, the animal should be treated using acaricidal washes or ivermectin (Ivomec Antiparasitic Injection for Cattle, ivermectin 10 g/L, Merck Sharpe and Dohme, or Ivomec Liquid for Sheep, ivermectin 0.8g/L, Merck Sharpe and Dohme) (See Note under 'Treatment Disclaimer').

There should be three consecutive negative faecal flotations. Heavy infections of coccidia, nematodes or cestodes should be treated appropriately. Any infestation of *Strongyloides spp* should be treated.

Faeces should be submitted for complete bacteriologic examination including routine culture, anaerobic culture, *Salmonella* culture, *Campylobacter* culture and ZN staining for acid-fast bacilli.

Macropodidae (kangaroos, wallabies, pademelons, potoroos and bettongs)

Health screening while in quarantine

Macropods should be kept in cat, fox and rodent-free enclosures for **60 days** Due to handling difficulties unnecessary testing should be minimised.

Serum should be tested for herpes virus, *Toxoplasma gondii*, and *Leptospira sp.* infection as per the methodology described above.

Faeces should be examined as for wombats and koalas for bacteria and there should be three negative parasite examinations. Faeces should be particularly examined for *Strongyloides spp* using a Baerman technique for larval separation.

Pre-release immunisations

Macropods should be vaccinated with a clostridial vaccine (Websters 5 in 1 Vaccine for Cattle and Sheep, Cyanamid Websters) twice four weeks apart. Vaccination with an ovine

foot rot vaccine (Footvax, Coopers) may provide immunity against necrobacillosis. While in quarantine Vitamin E powder should be added to the diet.

Pre-release treatments

Heavy burdens of coccidia and nematodes should be treated appropriately. Care must be taken when using some of the Benzimidazole drenches. This author feels that ivermectin (Ivomec Liquid for Sheep, ivermectin 0.8g/L, Merck Sharpe and Dohme) is the most appropriate drug for helminth parasites of macropods.

Burramyidae, Petauridae, Phalangeridae, Tarsipedidae and Pseudocheiridae (Possums and gliders)

Health screening while in quarantine.

The quarantine period is **60 days**.

Faecal examinations are as for macropods. In addition faeces should be cultured for *Yersinia pseudotuberculosis*. Serum should be tested as for macropods, and *Leptospira sp* may be a significant pathogen. It may be appropriate to test for *Cryptococcus sp* if it has been seen within the group and/or if it has been isolated from the environment, food trees or browse.

Peramelidae and Thylacomyidae (bandicoots and bilbies)

Health screening while in quarantine

The quarantine period is **60 days**.

Faeces should be tested for acanthocephalan parasites using direct smears, as the eggs of these parasites are difficult to detect using standard flotation and sedimentation techniques. *Echinonema sp* were found to be particularly pathogenic nematodes in Southern Brown bandicoots (*Isodon obesulus*) in Western Australia (Haigh, unpublished, 1994). Ziehl Neelsen staining of faecal smears may be particularly important in this group.

Dasyuridae (carnivorous marsupials)

Most of the information below refers to the Western quoll (*Dasyurus geoffroii*)⁸ (Haigh et al, 1994). Much of it will also be applicable to dunnarts, *Antichinus sp*.etc.

Health screening while in quarantine

Testing and a quarantine period of **60 days** are as for possums and gliders.

Faecal flotations for gastric and intestinal nematodes should be carried out. Strongyles are common, while coccidia are uncommon. Positive *Campylobacter sp* and *Yersinia sp* cultures and positive Ziehl Neelsen staining are significant in this species. *Salmonella sp.* is a common isolate and may not be significant.

Serology for *Leptospira interrogans*, *Toxoplasma gondii*, and *Chlamydia psittaci* is recommended. Methodology as above.

Myrmecobiidae (numbats)

Health screening while in quarantine

Numbats (*Myrmecobius fasciatus*) should have a **90 days** quarantine period. While in quarantine they should be fed termites known to come from areas where acanthocephalan parasite infections have not been seen in numbats. Numbats should be treated with ivermectin (Ivomec Antiparasitic Injection for Cattle, ivermectin 10 g/L, Merck Sharpe and Dohme) three times at monthly intervals, or twice, at the beginning and end of the quarantine period.

Blood samples should be taken to screen for regenerative anaemia, which has been seen commonly in captive numbats (Haigh, unpublished). Faeces should be cultured as for wombats. Particular attention should be paid to the presence of acid-fast bacilli in faeces. Cultures should be negative for *Salmonella*, *Campylobacter* and *Clostridium perfringens*, (Haigh, unpublished). Strongyle eggs and coccidia are common in wild numbat faeces (Haigh, unpublished).

Serology for *Leptospira interrogans*, *Toxoplasma gondii*, and *Chlamydia psittaci* is recommended. Methodology is as above.

MONOTREMATA

Ornithorynchidae (platypus)

Disease screening while in quarantine

Monotremes should be isolated in quarantine, confined in an appropriate enclosure for **90 days**.

Diseases that have been seen include *Mucor amphibiorum* skin infection, adenovirus infection, *Leptospira interrogans* infection, and *Aeromonas hydrophila* and *Salmonella sp* infection.

Blood smears should be screened for the red blood cell protozoan parasite *Theileria ornithorhynchi* and plasma should be checked for trypanosomes. Treat with Trivetin (Jurox, Trimethoprim/Sulphonamide 40mg/ml/200mg/ml) 5mg/kg I/M to reduce the shedding of coccidial oocysts in faeces.

Serum should be screened for *Toxoplasma gondii* antibody.

Treat heavy burdens of *Ixodes sp* ticks with ivermectin (Ivomec Antiparasitic Injection for Cattle, ivermectin 10 g/L, Merck Sharpe and Dohme) S/C at 200ug/kg as they are probable vectors of *Theileria*.

Tachyglossidae (echidnas)

Health screening while in quarantine

The quarantine period should be **90 days**.

Enclosures should exclude the definitive hosts of *Spirometra eranacei* (dogs, dingoes, cats and foxes).

Bleed to screen for anaemia. *Theileria*, *Babesia* and *Anaplasma* have occurred in clinically normal animals. Treat for coccidia prophylactically while in quarantine. The following potential disease agents have been seen: herpes virus, adenovirus, *Salmonella sp.* *Mycobacterium sp* has caused fatal generalised disease. *Microsporium gypseum* has been seen in echidnas with broken spines.

Three faecal floats should be negative for large numbers of nematodes. Treat ticks with ivermectin (Ivomec Antiparasitic Injection for Cattle, ivermectin 10 g/L, Merck Sharpe and Dohme) injection as they may be associated with dermatitis and anaemia.

Poisonous plants

Marsupials from the East Coast of Australia that are known not to be resistant to the Sodium fluoroacetate-bearing *Gastrolobium sp* and *Oxylobium sp.*, plants, which occur predominantly in Western Australia, should be released into areas that are free from these plants.

Chiroptera (Bats)

The following information is taken from personal communication with Dr. Hume Field of the Department of Primary Industries, Animal Research Institute, Marooka, Qld 4105, Australia.

Megachiroptera-Pterapoidae (flying foxes, fruit bats, blossom bats)

Screening of bat populations in the Australasian region is on-going. In wild populations of pteropids evidence of infection with Hendra virus (equine morbillivirus), Australian bat lyssavirus (ABL), Menangle virus and unidentified flavivirus/es (Australian and Papua New Guinean bat populations) have been found. A newly described paramyxovirus (Nipah virus) has recently been found in Malaysian bat species.

Microchiroptera (insectivores)

Some species have evidence of infection with ABL.

Health screening tests for Chiroptera

(Most tests are available through the Animal Research Institute, see address above).

Serum neutralisation tests (SNT) are used as the gold standard diagnostic test for Japanese encephalitis, Hendra virus, Menangle virus and Nipah virus. A fluorescent antibody test (FAT) is used to screen for ABL antigen in brain and salivary gland tissues and a rabies rapid fluorescent focus inhibition test is used to identify circulating antibodies to ABL. A competitive ELISA is used to screen for antibodies to the flavivirus serogroup prior to a specific SNT for Japanese encephalitis.

To the author's knowledge no viruses that have not been seen in the wild, have been detected in captive bats. Screening of captive bats prior to release or translocation would involve testing the recipient population and the bats to be translocated for the above viruses, and attempting to release naïve bats into virus free wild populations or positive bats into similar positive populations.

Pre-release immunisations

No controlled trials have been conducted on bat species. However, vaccination of bats destined for release into wild populations will potentially confound the interpretation of sero-surveillance of wild populations for evidence of natural infection and should therefore be avoided (Hume Field, pers. comm.).

Dr. Christopher Bunn (Commonwealth DPIE) has produced a document for the Australian Veterinary Plan on disease strategy for ABL in domestic animals and captive bat populations.

MARSUPIALIA (NEW WORLD)

Opossums (*Didelphis virginiana*)

Opossums in North and Central America appear to have a very low prevalence of rabies when compared with eutherian mammals (e.g. coyotes, foxes, skunks and raccoons).

Sarcocystis infestation may be a significant translocation concern. Opossums are the primary hosts for the sarcosporidian (*Sarcocystis neurona*) that causes Equine Protozoal Myelitis and are implicated in the spread of this disease. Opossums are also implicated in the occurrence of this neurological disease in captive cervids (e.g. white-tailed deer). Unfortunately, at present there is no serological test that could be used in a quarantine evaluation for this protozoon. If translocation of New World opossums to a “sarcosporidia-free” area is proposed, these facts should be borne in mind.⁹

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PHARMACEUTICAL COMPANIES AND LABORATORIES MENTIONED IN THE MARSUPIALIA CHAPTER

Commonwealth Serum Laboratories Ltd., Parkville, Victoria 3052, Australia.

Jurox Pty. Ltd., Unit 22/Block B, Slough Business Park, Holker St., Silverwater, N.S.W 2128, Australia.

Cyanamid Websters Pty Ltd., 23 Victoria Ave, Castle Hill, N.S.W 2154, Australia.

Intervet (Australia) Pty Ltd., Unit 3, 4 Gladstone Rd, Castle Hill, N.S.W, Australia.

Mallinckrodt Veterinary (Coopers Brand), 9 Ferntree Pl, Notting Hill, Victoria, 3149 Australia.

Merck Sharpe and Dohme, (Aust). Pty. Ltd, 54 Ferndell St, South Granville, N.S.W. 2142, Australia.

Mount Pleasant Laboratories, Department of Primary Industries and Fisheries, Kings Meadows, Tasmania, Australia, c/o Dr. David Obendorf.

TREATMENT DISCLAIMER

The drugs listed in the above report are not registered for use in Australian native species. They may therefore result in undesirable reactions or even death.

The mention of a specific drug vaccine or manufacturer by name in this text does not imply a recommendation by the author, editor and publishers but is merely a suggestion of what is available and where it can be found. Always consult the manufacturers and wildlife specialists before using these drugs.

It is recommended that a Veterinary Surgeon obtains and administers any Schedule 4 drugs in accordance with the national Veterinary Surgeons Act and the Poisons Act.

Note: There have been occasional problems when administering Ivomec Antiparasitic Injection for Cattle to some native Australian species. This author therefore recommends using the oral sheep preparation where possible. With echidnas, it is possible to give oral medications at the end of gas anaesthesia via a catheter and syringe, just as the animal is waking up and its mouth can be opened.

AVES

Health screening while in quarantine

Quarantine should last for **30 days**. But remember that some small species may die if closely confined for as little as 24 hours. Birds at high risk of Newcastle Disease should be quarantined for **60 days**.

The following are recommendations for appropriate testing procedures for diseases of birds while held in quarantine. When large numbers of birds of the same or related species are held as a flock or contiguous group, a series of random faecal samples should be examined. The serology screen should be appropriate^{1,2,3} for the disease profile of the species concerned.

1. Faecal examination, direct and flotation, for trichomonads, other motile protozoa and coccidia, gizzard worms of ducks and geese and tapeworms in small passerines. Stain faecal smear (Gram) and examine for *Candida sp.* and *Clostridia sp.*, stain faecal smear (Ziehl-Neelsen) for acid fast bacteria but remember that *Mycobacterium*

avium may be shed only sporadically and that thus a negative smear is not significant. For the diagnosis of avian tuberculosis a liver biopsy may be more useful.

2. Check for ectoparasites, especially *Amblyomma sp.* ticks that can be vectors of coudriosis-heartwater. If present, treat with an acaricide.
3. Carry out appropriate serological tests for chlamydiosis (psittacosis) and if positive, confirm by cloacal swab cultures. If culture is positive, the bird must be treated if of conservation value or destroyed if not. Treatment, which in some countries is mandatory for psittacine birds in quarantine, is lengthy, consisting of **45 days** continuous feed medication with chlortetracycline.

Note: Chlamydiosis (Psittacosis /ornithosis) is a dangerous zoonosis.

4. Faecal cultures for *Salmonella sp.* and *Campylobacter sp.*
5. Collect samples (choanal and cloacal swabs) for virus isolation from all incoming birds. Samples may be pooled from members of a flock. Samples for virus isolation should be routinely collected from all birds, which die in quarantine. All virus isolation tests should be negative in birds destined for release or entry into captive breeding flocks.
6. Carry out complete Blood Count and PCV.
7. Carry out serology/ELISA, as appropriate, for aspergillosis, *Chlamydia sp.*, paramyxovirus 1 (PMV-1), PMV-2, PMV-3, Eagle herpes virus, pigeon and raptor viruses, adenovirus, avian pox, avian influenza, mycoplasmosis and, for psittacines, "beak and feather virus" disease, Pacheco's disease and polyoma virus disease. All ELISA tests should be negative in birds for release or entry into captive breeding flocks.
8. Check raptors, *Otididae* (bustards) and *Columbidae* for oral trichomonosis.
9. Examine blood smears for avian malaria, *Babesia sp.* and *Leucocytozoon sp.*
10. Carry out endoscopy for aspergillosis in rare and valuable species.

Note: Birds destined for release into a Newcastle disease-free area should not originate from an area where Newcastle disease is present.⁴

Vaccination of exotic avian species against Newcastle disease is not recommended.

Newcastle disease has zoonotic implications. Clinical signs reported in humans are mild to severe conjunctivitis, general malaise and sinusitis, which usually resolve after 7-20 days. The wearing of goggles, gloves and a facemask when performing a postmortem examination on a bird is recommended.

Note: Translocation and re-Introduction projects for birds of all species, but especially psittacines, must always include very strict health screening and disease detection

measures. Some veterinary authorities are strongly opposed to the use of birds bred or held in captivity for re-introduction projects, believing that the disease risks are too great. Where possible, the birds should not leave their country of origin and captive-bred birds should not be exposed to non-indigenous species.

Birds bred or maintained for long periods in captivity may, like some mammals, develop behavioural aberrations that can interfere with their ability to mate, brood eggs and rear young. Such birds should be excluded from re-introduction programmes.

Of special concern is the health risk posed to wild populations of psittacines by diseases acquired by birds bred in aviaries or kept in captivity and which may become the subject of release projects. Birds maintained in captivity may be exposed to multiple diseases that may or may not be exotic to their native habitat.

The main diseases that are carried by captive psittacines and could seriously threaten wild psittacine populations (and some other species) if inadvertently introduced include:

Polyoma Virus

This viral disease is a threat to neonatal New World and some Old World psittacines. The virus may be a commensal infection of Australian cockatoos. It can cause very serious losses in New World species such as macaws. A PCR probe exists and there are known to be occult and intermittent excretors of the virus.

Psittacine Circovirus (Pbfd) or Psittacine Beak and Feather Disease

This virus primarily affects Old World and Australian species. Infection results in immunodeficiency and death. It is believed that the virus may have been introduced into Australia from Africa by aviary-bred birds. A PCR probe exists but may not identify all carriers.

Psittacine Herpesvirus (Pacheco's Disease)

This disease affects both New and Old World species. It is enzootic in South America and was first described in Brazil. Pacheco's Disease can produce very severe losses in birds stressed by crowded confinement. PCR probes are under development.

Chlamydia, Psittacosis or Ornithosis

This is a common rickettsial disease that can affect all bird species and some other vertebrates, including humans. Carrier status may be difficult to detect but PCR probes and serological tests exist. Treatment is mandatory in some countries (see above).

The following diseases create asymptomatic carriers for which **no known tests exist** at present. All are extremely dangerous.

Proventricular Dilatation Disease (PDD)

This disease can affect all species of psittacines and many non-psittacine species such as passerines and rhamphastids. It is believed to be viral and causes

irreversible neural damage to birds of all ages. The carrier state cannot yet be detected.

Papillomatosis

This disease is believed to be of viral origin and affects both New and Old World birds. The disease causes verrucose fleshy growths on the mucosae and can affect general health and reproductive capacity.^{7, 8, 9}

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It has been stated that most translocation projects involving amphibians and reptiles have not been successful and that they should not be advocated as conservation techniques. Consequently, if and when it is decided to release confiscated or unwanted pet reptiles into the wild, it is particularly important to screen them carefully, so as to minimise the risk of introducing a dangerous infection into the wild population.¹

Health screening while in quarantine

Quarantine for reptiles should last for **90 days**. Quarantine facilities should be adequate for the thermal requirements of the species under quarantine and allow for thermo-regulatory behaviour.

1. Faecal examination, direct and flotation, for protozoan⁶ (especially *Cryptosporidia sp.*⁴ and *Amoeba sp.*) and metazoan parasites. Three or more consecutive tests should be negative.
2. Culture faeces for *Salmonella sp.*

Note: Since > 80% of reptiles can test positive for *Salmonella sp.* evidence of infection may not preclude release.

3. Carry out complete Blood Count and PCV.
4. Examine blood smears for haemoparasites.
5. Swab/nasal wash and examination for *Mycoplasma sp.* and *Mycobacteria.sp.*

Note: Check the wild, recipient chelonian population for enzootic mycoplasmal infection, too. If the wild population is already infected, it may be unnecessary to disallow the release of infected chelonians.

6. Serology for antibodies of *Mycoplasma sp.* and herpes virus for chelonians (especially *Testudo hermanni* and *T. graeca*) and orphidian paramyxovirus for reptiles^{2,5}.
7. Check for tick infestation, especially *Amblyomma sp.* that can be vectors of cowdriosis-heartwater. If present, treat with an acaricide.

Note: Green sea turtles (*Chelonia mydas*) which have been treated surgically for fibropapillomatosis should be released only in the area in which they were found, since this condition seems to occur in certain defined geographic areas.

Crocodylia

(Compiled by F. Huchzermeyer,³ IUCN/SSC Crocodile Specialist Group).

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Pre-release quarantine for farm-bred crocodylia, beyond the time required to carry out a clinical examination, apply specific serological tests and where appropriate, pre-release treatment for parasitic worms, is considered to be stressful and unnecessary.

Wild populations are often reservoirs of the known crocodile-specific diseases: caiman pox, crocodile pox, adenoviral hepatitis, chlamydiosis, mycoplasmosis (arthritis) and coccidiosis.

Most of the specific diseases of crocodylia listed above are enzootic in wild populations and wild crocodiles thus present a greater danger to captive (farmed) crocodiles than the reverse.

Unfortunately, except for chlamydiosis, there are no serological tests for the above diseases. Tests for mycoplasmosis might be possible if the relevant test antigens could be prepared. Pre-release testing for chlamydiosis in Nile crocodiles and clinical examination for mycoplasmosis in Nile crocodiles and American alligators should be carried out as a routine.

Crocodile coccidial oocysts are extremely fragile and are rarely detected in faecal flotations or direct smears. However, coccidiosis is considered to be a major disease of farmed crocodiles in Zimbabwe.⁸

The examination of blood smears from crocodiles serves little purpose since the known blood parasites of crocodiles appear to be harmless.

Gastrointestinal nematode infections are usually asymptomatic in crocodiles but may occasionally be associated with disease. Infection with *Dujardinascaris sp.* may cause disease and can be associated with gastric ulceration and runting in hatchlings.^{8, 11}

Nematode larvae in the muscles of slaughtered Nile crocodiles from farms in Zimbabwe have been tentatively identified as *Trichinella spiralis nelsoni* but this identification has since been questioned and it has been suggested that they may represent a new taxon of *Trichinella*. The distribution of *T. spiralis* is cosmopolitan and it can transmit to domestic livestock and humans.⁹

Pentastomes often occur in crocodylians in great numbers, both as adults and also as larvae and nymphs. Pentastomiasis is regarded as a major disease on crocodile farms in Australia.¹⁰

Pre-release clinical examination of crocodiles should exclude cases of dermatitis.

If farmed crocodylians are to be released into the wild, a health certificate covering the farm of origin and a clinical examination of the animals to be released should be obtained. No runts, animals in poor condition or with visible abnormalities should be released.

Crocodiles from farms with a recent history of disease and mortality should not release animals into the wild and juvenile crocodiles which have been fed on river fish should be treated with an anthelmintic before release.

When possible, release should not take place in winter or during the cold months of the year.

The release area should be ecologically correct for the age group to be released and the behaviour and movement patterns of the species concerned should be considered.

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For a full account of the diseases of crocodiles See: *Import Risk Analysis Paper for Live Crocodilians and their Eggs*. January, 2000. Australian Quarantine and Inspection Service (AQIS), GPO Box 858, Canberra ACT 2601, Australia

AMPHIBIA

(Compiled by A. A. Cunningham,⁴ P. Daszak⁵ & A. D. Hyatt⁶)

Infectious disease threats to amphibians

Threats to wildlife of infectious disease entities brought about by anthropogenic factors are being increasingly recognised.^{1, 2} Although increasing at a rapid rate, knowledge of the identity and epizootiology of infectious diseases that affect amphibians is relatively poor. However, a number of diseases has been recognised which could potentially be devastating should they be introduced to naïve populations. Two of these, amphibian chytridiomycosis and ranavirus disease, have recently emerged as major threats to the survival of wild amphibians on a global scale, possibly via the activities of humans.³ Although further investigations are required before such hypotheses are substantiated or refuted, it would be wise and prudent to ensure measures are taken to minimise the threats of introduced disease when working with, and in particular when translocating, amphibians, regardless of the purpose of the work.

As with all animals, when considering the translocation of amphibians, both the source and destination of the animals must be taken into account. The longer an animal is maintained in captivity, for example, the greater the chance it will have an altered complement of symbiotic and parasitic flora and fauna to that found in its natural habitat. The ultimate goal of screening animals prior to translocation is to prevent the co-introduction of alien organisms and to maximise the chance success rate of the project. As it is impossible (through lack of knowledge, funds, *etc.*), and probably impractical even if possible, to ensure this is done to a final conclusion, pragmatic alternatives have to be taken.

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Quarantine period and general screening to determine suitability for release

Animals to be translocated should be quarantined, either prior to shipment, in a holding area on arrival, or preferably both, prior to release. The time of this quarantine period is arbitrary given the lack of knowledge of amphibian diseases, but should certainly be no less than 30 days.

During this holding period, every animal should be examined for obvious signs of ill-health. The presence of ill-health (presence of lesions, poor body condition, *etc.*) automatically renders an animal unfit for release on welfare grounds.

Animals should be examined for subclinical presence of parasites (taken here to include eukaryotic organisms, prokaryotic organisms and viruses). The presence of parasites does not necessarily rule out animals for release, provided the parasites present are naturally enzootic to the area of release. If there is a large number of animals, it may not be necessary to examine each animal for evidence of parasites, provided a statistically meaningful number are examined from each batch within parasite-transmission contact (defined as contact close enough for the transmission of a specific parasite to occur between hosts and for a long enough time period, *e.g.* pre-patent period for certain nematodes, to enable such transmission to be detected).

Any animal that dies during the pre-release quarantine period must be necropsied and examined for evidence of disease, including specific histopathological examinations and culture for iridoviruses and cutaneous chytrids.

Measures should be taken, within reason, to prevent the release of animals into an area where disease to which they are not immune is enzootic. There should, therefore, be some knowledge of the parasite status of animals in general, and amphibians in particular, at the release site, for example by conducting necropsies on animals found dead or killed (such as those hunted/fished) in the area. If animals of the same species are already present at the site of release then, if possible, a statistically meaningful number should be examined to enable a reasonably accurate picture to be gained of the endemic parasite flora and fauna. The presence or absence of ranaviruses and cutaneous chytrid fungi, in particular, must be determined prior to the release of the translocated animals.

Given the dangers of potentially catastrophic epizootic ranavirus disease or cutaneous chytridiomycosis, animals harbouring these organisms must not be used for translocation. Sites where evidence of ranavirus disease or cutaneous chytridiomycosis are found must not be used for the release of amphibians. There are many different types of amphibian ranavirus and this may also be the case for amphibian chytrid fungi. Therefore, even where evidence of such a parasite is found in both translocated animals and release sites, it is strongly recommended that caution be erred upon and no release be conducted.

Finally, it should be remembered that the alteration of the exposure to parasites following the release of translocated animals can have unforeseeable consequences, including harmful effects on genera, orders or classes other than those of the target animals.^{1,2}

Minimum screening required

These procedures should be carried out as indicated above for live animals destined for translocation and, where possible, during necropsy of animals that have died during the translocation period, or those collected from target release sites.

No immunisations are currently available for ranavirus disease, cutaneous chytridiomycosis or other significant infectious diseases of amphibians.

1. Cutaneous chytridiomycosis

Diagnosis is by identification of characteristic intracellular flask-shaped sporangia and septate thalli within the superficial epidermis.^{4, 3, 5} The most reliable technique is histology, either of a toe-clip taken from a live animal, or of toe-clips and ventral skin (from the pelvic “drink” patch) taken from a necropsied animal. Full protocols for examination and histology are given in a web-published article,⁶ available at the “Amphibian diseases home page” run by R. Speare of the James Cook University, Australia (<http://www.jcu.edu.au/dept/PHTM/frogs/ampdis.htm>). Wright’s- or Diff-Quik- (Difco Laboratories, Detroit, Michigan, USA) stained smears of skin scrapings⁵ or impression (touch) smears of ventral pelvic (“drink”) patch skin stained with Wright’s or Diff-Quik are also potentially useful, however smears are less reliable than histologic analysis. Research is currently underway to develop ELISA and other antibody-based tests and PCR-based tests; polyclonal antibodies against chytrids (not *Batrachochytrium dendrobatidis* specific) are available from the Australian Animal Health Laboratory (Geelong Australia) together with an immunoperoxidase protocol.

2. Ranaviruses

Animals exhibiting lesions or clinical signs consistent with the range observed in ranavirus disease of anurans and urodeles^{7, 8} should be necropsied and viral presence determined by culture in commercially available cell lines. Due to differential culture characteristics of various ranaviruses, a range of cell lines, including fish and amphibian cells, should be used. Cell lines in which ranaviruses have been successfully cultured include fathead minnow (FHM) epithelial cells (European Collection of Animal Cell Cultures No. 88102401), *Rana pipiens* embryo fibroblast cell line (ICR-2A, ECACC), epithelioma papulosum cyprini cells (EPC cells, Life Technologies, Grand Island, New York, USA), Chinook Salmon Epithelial (CHSE) cells and Vero cells. Culture should be conducted at between 25 and 27 °C as this appears to be the optimum range for ranavirus growth. Ranaviruses do not grow at temperatures above 30°C. The cytopathic effect (CPE) produced by ranaviruses depends on the virus species and the cell culture used, but typical ranavirus CPE in cell monolayers consists of discrete, progressive plaques of rounded-up and sloughing cells. Details (cells, temperature and procedures) for the isolation of ranaviruses can be found in the Office International des Epizooties (OIE) "*Diseases Manual for Aquatic Animal Diseases*".

Virus can be identified directly in tissues or in cell cultures by electron microscopy with the examination of ultra-thin sections and the examination of negative-stained particulate samples.^{9, 10} Unfortunately, no general serological test has yet been evaluated for the detection of antibodies within susceptible animals. Specific antibody detection assays exist for *Bufo marinus* and these assays can be adapted to a general competitive ELISA but the sensitivity and specificity of the latter is not known. A large number of ranaviruses have now been examined¹¹ and the data show that the OIE accepted EHNV antigen-capture ELISA¹¹ can be used to detect all known ranaviruses. Further details of these ELISAs are available from Dr Alex Hyatt, CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia. Ranaviruses can also be detected (*in-vivo* and *in-vitro*) by PCR.^{12, 13}

3. Erythrocytic iridoviruses

These can be identified by light microscopy of blood cells on air-dried, Giemsa-stained blood smears, with follow-up electron microscopy if intracellular inclusions are found.

4. Enteric and pulmonary helminths

The presence of helminth eggs or larvae can be detected using standard methods for light microscopical examination of wet faecal smears.

5. Enteric protozoa

Enteric protozoa can be detected using light microscopy of wet faecal smears. It should be remembered that a range of commensal, and possibly also symbiotic, protozoa may be found using this technique, in addition to parasitic organisms.

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FISH

(Compiled by F. Scullion)⁷

Wild fish releases

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There are unquantifiable disease risks for releases involving wild fish in direct translocations. Although these need to be reflected in stringent quarantine requirements, quarantine by itself, in such circumstances, can only act as an aid to disease prevention and should not be considered as a guarantee. Full consideration should be given to the need for any such movement and if deemed necessary it is essential to develop a comprehensive veterinary risk assessment for each specific case, with quarantine protocols directed at identified specific risks, in consultation with local veterinary expertise.

Captive releases. Salmoniformes

Salmonid fish have been bred in captivity for release in rehabilitation programmes for over a century.

The following are the minimum recommendations for appropriate testing procedures of captive-bred salmonids for the major diseases of salmonid fish held in quarantine prior to release. These requirements should be applied in addition to those imposed by adherence to guidelines such as of the International Aquatic Animal Health Code produced by OIE.

General health measures

Fish selected to enter a pre-release quarantine facility are best sourced from a facility with good quality veterinary health programmes in place. Prior disease history should be thoroughly evaluated before fish are entered into any release programme.

Further, any disease problems encountered during the quarantine period should be thoroughly investigated by the quarantine facility's veterinarian and decisions regarding release should include the evaluation of the results of such investigations as well as pre-entry disease history. Parasites - Gill squash, skin scrape and faecal examinations should be carried out to establish the major ecto- and endo-parasites associated with the fish. Treatment for identified parasites should be applied within the quarantine period in accordance with local veterinary advice.

Viral diseases

The recognised means of preventing spread of fish viruses is to avoid the contact of fish and virus. This is best done when fish are sourced from areas recognised as being free from these diseases.

Bacterial diseases

Bacterial kidney disease - Fish for release should be sourced from an area recognised as free from this disease.

Furunculosis and Enteric Redmouth - Vaccination is recommended (use an oil adjuvant based vaccine for furunculosis). Time should be allowed within quarantine to include development of sufficient protection before release, in accordance with the manufacturers' instructions.

Fungal diseases

A number of species of fungi are associated with disease processes in fish. However, although fungi are infectious agents the aetiology of many fungal diseases includes stress (including normal physiological endocrine-driven changes in some instances), and environmental, husbandry and management factors, as predisposing causes. Many fungal agents are ubiquitous in nature and prevention of disease-spread during fish movements is directed at prior attention to optimum husbandry of fish in captivity, minimising stressors, culling and/or strategic therapy, as advised by local veterinary expertise.

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Sources of Vaccines and Pharmaceuticals

Note: The mention of the names of pharmaceuticals, vaccines and their manufacturers in this publication does not imply recommendation. It is merely a guide as to what products are available and their sources. Many of the drugs and vaccines mentioned here are not licensed for use in wild animals and their safety and efficacy are untested. In addition, veterinary drugs and vaccines are continuously undergoing development and improvement. Veterinarians are therefore strongly advised to consult manufacturers and specialists before using the products mentioned in this booklet.

It is important to remember that veterinary pharmaceuticals and vaccines are produced and marketed worldwide and may be manufactured and distributed in different countries under different names. The head office of the manufacturer may be in the U.S.A. or Europe or elsewhere and will always advise on the availability of products in other countries.

- ❖ Merial Ltd. (formerly Rhone Merieux Inc.)
115 Trastech Drive,
Athens, GA 30601
United States of America Tel: 1(800) 255 6144 or 1(888) 637 5251

Imrab3 (Killed Rabies Vaccine)
Eprinomectin (Eprinex) (ruminant anthelmintic)
Fipronil, Frontline, Top Spot and Spray (Ectoparasite control)

- ❖ Butler,
4344 Federal Drive, Suite 104,
Greensboro, NC 27401
United States of America Tel: 1(800) 551 3861 Fax: 1(888) 329 3861

Various canine, feline, equine, bovine and porcine vaccines

- ❖ Pfizer Animal Health Pfizer Ltd.
PO Box 747029 Ramsgate Road, Sandwich
Pittsburgh, PA 15274-7029 Kent CT13 9NJ, U.K.
United States of America Tel: 1(800) 733-5500

Leptoferm 5, BratiVac-6 (Leptospira vaccines)
Doramectin (Dectomax) (Ruminant anthelmintic)

- ❖ Bayer Corporation, Animal Health
PO Box 751772
Charlotte, NC 28275
United States of America Tel: 1(913) 631-4800

Vision 7, Vision 7 with SPUR (Clostridial bacterin-toxoid for all ungulates)
Super-Tet with Havlogen (Tetanus vaccine)
Prestige with Havlogen (Killed equine herpes virus vaccine)

Imidacloprid, Advantage (Ectoparasite control)

❖ Fort Dodge Animal Health
9401 Indian Creek Parkway
Ste. 1500
Overland Park
KS 66210
United States of America

Fort Dodge Animal Health
Flanders Road
Hedge End
Southampton SO30 4QH
U.K.

Triangle 3 (combined IBR, BVD and P13 vaccine)

Cydectin (Moxidectin) (ruminant anthelmintic)

Fluvac (Equine influenza vaccine)

Encephaloid (Killed EEE and WEE vaccine)

Fel-O-Vac PCT and Fel-O-Vac IV (3-way killed vaccine for feline rhinotracheitis, calicivirus and panleucopaenia)

Duramune KF-11 (Modified live canine parvovirus vaccine)

Fel-O-Vac (Inactivated)

Duvaxyn IE and IET

(Equine Influenza and tetanus vaccines)

❖ SmithKline Beecham Animal Health
West Chester
PA 19380
United States of America

Albendazole and Valbazen (Flukicides)

❖ Merck Agvet
Division of Merck and Co. Inc.
P.O. Box 2000
Rahway, N.J. 07065
United States of America

Clorsulon and Curatrem (Flukicides)

Ivermectin (Ivomec) (ruminant anthelmintic)

❖ Schering-Plough Animal Health Corporation
P.O. Box 3113
Omaha, NB 68103
United States of America

Permethrin 11 (Pyrethrin acaricide)

❖ Colorado Serum Company
4950 York Street
Denver, CO 80216
United States of America

Anthrax vaccine

❖ Haver-Lockhart
Shawnee

KS 66201
United States of America

Praziquantil (Droncit) (Intramuscular anthelmintic for Echinococcosis)

- ❖ Biocor, Pharmacia and Upjohn
7000 Portage Road
Kalamazoo, MI 49001
United States of America

Adenomune-7 (Live vaccine for canine infectious hepatitis)

- ❖ Intervet U.K.Ltd.
Science Park
Milton Road
Cambridge CB4 0FP
U.K.

Nobi-Vacc Lepto (Inactivated vaccine containing *Leptospira canicola* and *L. Icterohaemorrhagiae* antigens)

- ❖ Hoechst Rousel Vet Ltd.
Walton Manor
Walton, Milton Keynes
Bucks. MK7 7 AJ
U.K.

Heptavac (Clostridial vaccine for Artiodactyla)

- ❖ United Vaccines Inc.
P.O. Box 44220
Madison
WI 53744-4220
United States of America

Vaccines for mink, ferrets and foxes

Two useful sources of relevant information are:

1. Soorae, P.S. and Seddon, P.J. (Eds). 1998. *Re-introduction Practitioners Directory*. Published jointly by the IUCN Species Survival Commission's Re-introduction Specialist Group, Nairobi, Kenya and The National Commission for Wildlife Conservation and Development, Riyadh, Saudi Arabia. 97pp.

Copies of the above Directory are available from: The Secretary-General, National Commission for Wildlife Conservation and Development, Post Box 61681, Riyadh 11575, Kingdom of Saudi Arabia. Tel.(966-1) 441 8700, Fax. (966-1) 441 0797.

2. American Zoo and Aquarium Association (AZA), *Reintroduction Guidelines*. Copies of these guidelines are obtainable from: Dr. Benjamin Beck, National Zoological Park, Smithsonian Institution, Washington, D.C. Email: bbeck@nzp.si.edu.

USEFUL ADDRESSES

This list of addresses that follows does not pretend to be comprehensive. Addresses and telephone numbers can change and where possible should be checked before use.

1. CSIRO Division of Wildlife and Ecology
P.O. Box 84
Lyneham, ACT 2602
AUSTRALIA
2. CSIRO Division of Animal Health
Long Pocket Laboratories
Private Bag, No. 3
Indooroopilly, QLD 4068
AUSTRALIA
3. Non-Domestic Register of Pathology
Taronga Zoo
P.O. Box 20, Mosman, NSW 2570
AUSTRALIA
4. Marine Animal Resources Centre
69 Main Street
Pialba, QLD 4655
AUSTRALIA
5. Chief Central Veterinary Laboratory
Frome Road
G.P.O. Box 1671, Adelaide, SA
AUSTRALIA
6. University of Melbourne Veterinary Clinical Centre
Princess Highway
Melbourne, VIC. 3030
AUSTRALIA
7. Animal Health Division
Dept. of Agriculture
Jarrah Road
South Perth, WA 6151
AUSTRALIA
8. Department of Agriculture

Mount Pleasant Laboratories
P.O. Box 46
Launceston, TAS. 7250
AUSTRALIA

9. Australian Association of Veterinary Conservation Biologists (AAVCB)
C/o The Australian Veterinary Association
134-136, Hampden Road
Artarmon, NSW 2064
AUSTRALIA
10. Institut National de Recherches Veterinaires
Groeselenberg, 99
B-1180 Bruxelles
BELGIUM
11. Centre de Medicine du Gibier
Universite de Liege
Faculte de Medicine Veterinaire
Sart-Tilman 4000 Liege
BELGIUM
12. Brazilian Association of Wildlife Veterinarians (ABRAVAS)
Fundação Parque Zoologico de São Paulo
Ave. Miguel Stefano 4241 Agua Funda
04301 São Paulo SP
BRAZIL
13. Asociacion Latinoamericana de Medicos Veterinarios en Fauna Silvestre
Edificio Batallon Colorados Piso 11 Apto 04
Calle Batallon Colorados N.24
Centro La Paz
BOLIVIA
13. National Wildlife Research Centre
Canadian Wildlife Service
Environment Canada
100 Gamelin Boulevard
Hull, Quebec, K1A 0H3
CANADA
14. Canadian Cooperative Wildlife Health Centre (CCWHC)
Department of Veterinary Pathology
Western College of Veterinary Medicine
University of Saskatchewan
Saskatoon, SK S7N 0W0
CANADA
15. Canadian Association of Zoo and Wildlife Veterinarians

- 2355 Corydon Avenue
Winnipeg
Manitoba R3P 0R5
CANADA
16. Central Veterinary Laboratory
Department of Veterinary Sciences
Athalassa, Nicosia
CYPRUS
 17. State Veterinary Institute
Department of Virology
Rantirovska 93
586 02 Jihlava-Horni Kosov
CZECH REPUBLIC
 18. National Veterinary Laboratory
2 Hangovej
DK-8200 Aarhus
DENMARK
 19. Veterinary Research Institute
Dokki
EGYPT
 20. Veterinary Research Laboratory
Department of Agriculture and Food
Abbottstown
Dublin 15
EIRE
 21. National Veterinary Institute
P.O. Box 19
Debre Zeit
ETHIOPIA
 22. Finnish Forest Research Institute
Department of Forest Ecology
P.O. Box 18
SF-01301 Vantaa
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 23. Institut Pasteur
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